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ORIGINAL ARTICLES

The Clinical Research Tool: A High-Performance Microdialysis-Based System for Reliably Measuring Interstitial Fluid Glucose Concentration

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Abstract

Background:

A novel microdialysis-based continuous glucose monitoring system, the so-called Clinical Research Tool (CRT), is presented. The CRT was designed exclusively for investigational use to offer high analytical accuracy and reliability. The CRT was built to avoid signal artifacts due to catheter clogging, flow obstruction by air bubbles, and flow variation caused by inconstant pumping. For differentiation between physiological events and system artifacts, the sensor current, counter electrode and polarization voltage, battery voltage, sensor temperature, and flow rate are recorded at a rate of 1 Hz.

Method:

In vitro characterization with buffered glucose solutions ($c_{glucose}$ = 0 - 26 x 10^{-3} mol liter^{-1}) over 120 h yielded a mean absolute relative error (MARE) of 2.9 ± 0.9% and a recorded mean flow rate of 330 ± 48 nl/min with periodic flow rate variation amounting to 24 ± 7%. The first 120 h *in vivo* testing was conducted with five type 1 diabetes subjects wearing two systems each. A mean flow rate of 350 ± 59 nl/min and a periodic variation of 22 ± 6% were recorded.

Results:

Utilizing 3 blood glucose measurements per day and a physical lag time of 1980 s, retrospective calibration of the 10 *in vivo* experiments yielded a MARE value of 12.4 ± 5.7. Clarke error grid analysis resulted in 81.0%, 16.6%, 0.8%, 1.6%, and 0% in regions A, B, C, D, and E, respectively.

Conclusion:

The CRT demonstrates exceptional reliability of system operation and very good measurement performance. The ability to differentiate between artifacts and physiological effects suggests the use of the CRT as a reference tool in clinical investigations.

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Abbreviations: (BG) blood glucose, (CRT) clinical research tool, (EGA) error grid analysis, (MARE) mean absolute relative error, (PAES) polyarylethersulphone, (PC) personal computer, (PRESS) predictive error sum of squares, (SCGM) subcutaneous continuous glucose monitoring, (SMBG) self-monitoring of blood glucose

Keywords: biosensor, clinical investigation, continuous monitoring, flow rate sensor, glucose, microdialysis

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Introduction

Self-monitoring of blood glucose (SMBG) systems are widely used by patients with diabetes to accurately determine capillary blood glucose (BG) values. As pointed out by several workers in the field,[1] even prohibitively frequent SMBG measurements may fail to correctly reflect daily glucose profiles, leaving lengths and occasional occurrences of glycemic excursions undetected. Minimally invasive glucose monitoring techniques with daily temporal resolution of 288,[2,3] 480,[4] and 1440[5] values have been developed. Devices for continuous glucose monitoring have demonstrated its usefulness for a plurality of diabetes patients such as children with type 1 diabetes,[6] patients with gestational diabetes,[7] as well as patients with type 1 diabetes who are experiencing nocturnal hypoglycemic events.[8–10] Continuous glucose monitoring devices have been utilized to record postprandial glucose levels[11] to make better informed therapy decisions.

While several different minimally invasive approaches have been pursued to realize a continuous glucose monitoring system (see References 12–15 for reviews), microdialysis and needle-type sensing have already extensively displayed their respective characteristics. Needle-type systems allow virtual elimination of instrument time lag, simple handling procedures, and small overall instrument size. While the system size and the handling of these systems are already quite advanced, the complex foreign body response to an *in vivo* sensor requires very significant additional development steps to meet International Organization for Standardization 15197 criteria.[16]

In contrast to needle-type sensors, microdialysis systems are employing flow-through *ex vivo* sensors continuously flushed with interstitial fluid extracts. Due to the good biocompatibility of catheters used for extraction as well as the diminished issue of sensor fouling, excellent analytical performance could be achieved.[17] While devices with a lag time of up to only a few minutes have been introduced,[18] presented,[19] and proposed,[20] the performance benefit was frequently contrasted with a very significant physical lag time, due to the large dead volume between microdialysis catheter and glucose sensor, as well as the low flow rate often chosen for reasons of maximizing glucose recovery.[5] Further, a comparably higher system complexity has to be taken into account, which leads to multiple handling steps, a comparably larger system size, and higher manufacturing cost. While these reasons may have prevented widespread use of microdialysis-based systems, other issues may have contributed as well. In order to provide high system accuracy, variation of mean flow rate has to be minimized and the introduction of air bubbles into the flow system has to be avoided. While bubble-related issues are extensively described in microfluidic literature,[21,22] adapted and novel solutions remained to be developed to prevent *inter alia* following effects specifically detrimental to the accuracy of microdialysis systems, such as the so-called SCGM 1 system.[5] In general, flow rate variation leads to inconstant physical lag time and thus to increased measurement error when a constant physical lag time is used prior to calibration of sensor signals. Flow rate variation further results in changes of glucose recovery in the microdialysis catheter[23,24] and altered sensor sensitivity,[25] while bubbles, trapped in the flow-through sensor, lead to highly variable sensor response due to incomplete wetting of sensing surfaces. To avoid these effects, constancy of mean flow rate through the system and complete removal of bubbles has to be established. Furthermore, to be able to assess the quality of each continuous measurement, it is beneficial to continuously record and indicate the flow rate. A novel microdialysis system, the so-called Clinical Research Tool (CRT), which was built to eliminate flow and bubble artifacts and to allow continuous indication of system condition, shall be described in this article. The subject of this report is the performance of the CRT demonstrated in the first *in vitro* and *in vivo* experiments.

Materials and Methods

System Description

The CRT is a microdialysis-based system with an electrochemical flow-through sensor, which relies on a constant mean flow rate through the catheter and through the sensor. Fluid propulsion is enacted via a push–pull pumping action of a peristaltic pump. The push and pull flow rates are matched carefully to minimize the contribution of convective mass transfer across the microdialysis membrane. The system is calibrated retrospectively using capillary BG measurements, converting current measurements (nA) into glucose concentrations (mg/dl). Hereto the BG concentration is measured with an ACCU-CHEK® Compact system (Roche Diagnostics, Germany). Subsequently, results are transferred to the CRT control personal computer (PC).

The CRT consists of four main components—the CRT instrument, the CRT control PC, the CRT disposable, and the CRT flow rate sensor—as depicted in **Figure 1**. The CRT instrument, the sterile CRT disposable, and the disinfected CRT flow rate sensor are joined prior to use, whereas the CRT control PC is connected with the instrument during start-up, control of ongoing experiments, and stop of data acquisition.

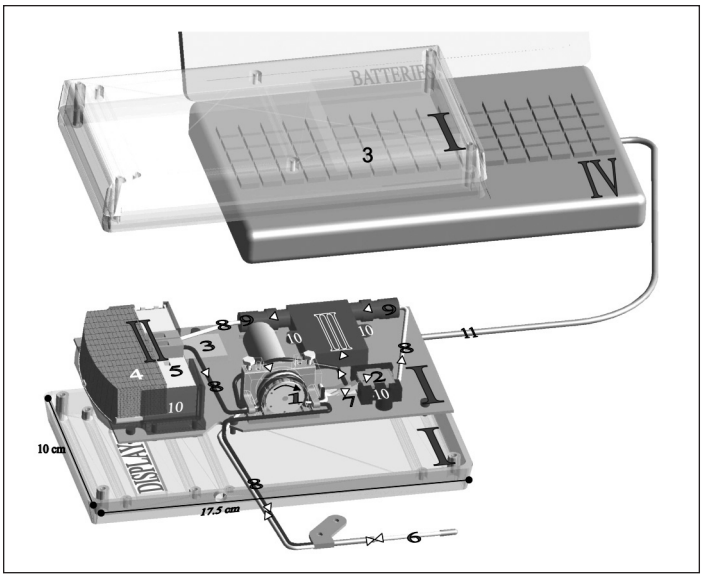

Figure 1. Exploded view of the CRT system. The system consists of an instrument (I), a disposable (II), a flow rate sensor (III), and a control PC (IV), which are connected prior to use by means of connectors (9), mounting clamps (10), and an interface cable (11) followed by closure of the shell case. The PC (IV) is connected with the instrument (I) during start-up, control of ongoing experiments, and stop of data acquisition, followed by data transfer from a data storage card (3) to the PC (IV). The sensor current, flow rate, and system alarms are displayed by the instrument (I). All system parameters are available by coupling the instrument (I) and the PC (IV). The disposable (II) consists of one pouch cassette (4) and one tubing cassette (5), which are packaged separately. The microperistaltic pump (1) continuously serves to propulse the perfusion liquid via connecting tubes (8), thereby withdrawing the perfusion liquid from the pouch cassette (4), directing it into the microdialysis catheter (6), withdrawing the dialysate from the microdialysis catheter (6), pushing the dialysate into the bubble filter (7), degassing the dialysate, and directing the dialysate to the glucose flow-through sensor (2), the flow rate sensor (III), and finally to the waste reservoir of the pouch cassette (4).

The CRT instrument contains a microprocessor that controls all system components, i.e., a microperistaltic pump, a potentiostat, and a temperature sensor located underneath the flow-through glucose sensor. Current data from amperometric measurements are recorded every second and stored on a data-storage card. Further, the following system parameters are recorded at a rate of 1 Hz and subsequently stored on a data-storage card: the battery voltage, the temperature of the glucose sensor, the voltage of the counter electrode of the flow-through sensor, the polarization voltage of the flow-through sensor, and the flow rate measured by the CRT flow rate sensor. The sensor current and all system parameters are displayed by the CRT control PC on demand when the CRT instrument and the CRT control PC are connected to each other. In addition, the sensor current and the flow rate as well as distinct and settable alarms triggered by underrun of settable flow rate thresholds, sensor-to-socket contact interrupts, low battery capacity, and data-storage card write errors are displayed in real time and recorded by the instrument. Instrument batteries are currently replaced every second day.

The CRT disposable resembles the SCGM 1 disposable[5] in that it consists of two disposable components, a modified pouch cassette, and a modified tubing cassette; the pouch cassette contains a reservoir bag with a maximum capacity of 10 ml sterile isotonic saline (1.47×10^{-1} mol liter^{-1} NaCl) and an empty reservoir bag. The sterile tubing cassette contains a microdialysis catheter (CMA 60, CMA Microdialysis, Stockholm, Sweden) with a nominal cutoff of 20 kDa and a water permeability of $5 - 8 \times 10^{-4}$ cm/bar/sec (T = 37 °C, p = 100 mbar), a built-in house assembly of a bubble filter attached to the inlet of a flow-through glucose sensor, connecting tubing, and connectors to the pouch cassette. The bubble filter features a flow-through hydrophobic hollow fiber surrounded by a chamber with reduced pressure, thus removing air from the perfusion liquid. The amperometric flow-through glucose sensor features a microchannel and a screen-printed three-microelectrode assembly consisting of a working electrode with immobilized glucose oxidase, an Ag/AgCl reference electrode, and a carbon counter electrode. The CRT flow rate sensor (Honeywell, X119177-AW, Freeport, Illinois) coupled to the outlet of the glucose sensor is used to monitor the volumetric flow rate of the dialysate. The flow rate sensor can be used in a continuous, programmed discontinuous, or on-demand mode if power consumption is to be minimized.

Procedures

Flow rate sensors were disinfected prior to each use of a CRT disposable. The pouch cassette, the tubing cassette, the flow rate sensor, and the CRT instrument were connected to each other immediately prior to use. The disposable was coupled to the instrument via mounting clamps to hold the disposable cassette and the flow rate sensor in place. The CRT control PC with Linux-based control software served to control the instrument, to provide online access to all data acquired,

to allow communication with BG meters used to acquire capillary blood measurements, and to create subject and experiment log files.

In Vivo Study

The first *in vivo* tests were conducted in compliance with the Helsinki Declaration at the Institute for Diabetes Technology at the University of Ulm, Germany. Five female type 1 diabetes patients participated in this study (mean hemoglobin A1c of 7.6% [5.9–8.9 range], mean age of 52 years [37–63 range], diabetes for 22 years (mean) [5–34 range]). The BG was measured at least 14 times per day with an ACCU-CHEK Compact BG meter (Roche Diagnostics, Mannheim), while at least 10 duplicate BG values were used for validation of system performance. In each subject, two CRT systems (investigational devices, Roche Diagnostics, Mannheim) were placed in the abdominal subcutaneous tissue. The CRT experiments contained 86,400 data points per 24 h over a time span of 120 h.

Results

Typical Data Set

In order to ensure system operation according to specifications, all relevant system parameters such as the counter electrode voltage, the battery voltage, and the system flow rate were continuously recorded by the CRT. **Figure 2** depicts a typical *in vivo* data set to illustrate the degree of system monitoring and the time resolution obtainable. **Figure 2A** shows the current of the amperometric glucose sensor over monitoring time. **Figure 2B** depicts the pulsation of the flow rate, which is induced by the squeeze and relaxation of the elastic tubing and is monitored by the continuously operating flow rate sensor over time. A highly periodic pulsation and highly constant mean flow rate was recorded, while the amplitude of pulsation was found to vary over time. The root cause of these amplitude changes are back-pressure changes, which originate from the increase in the fluid volume stored in the waste pouch, changes of the cross section of the connecting tubing due to movement, as well as from bubbles entering the fluidic system. Due to the composition of the working electrode itself, short-term flow rate pulsations, such as those caused by the peristaltic pump action, were not detectable by the glucose flow-through sensor. However, events such as slow displacement of air off the inner surface of the microdialysis catheter, passage of bubbles through the system, and subsequent removal of bubbles from the bubble filter were found to be apparent from a zero flow rate during the time of bubble removal and short-term sensitivity increase of the glucose sensor response. The counter electrode voltage was recorded to monitor

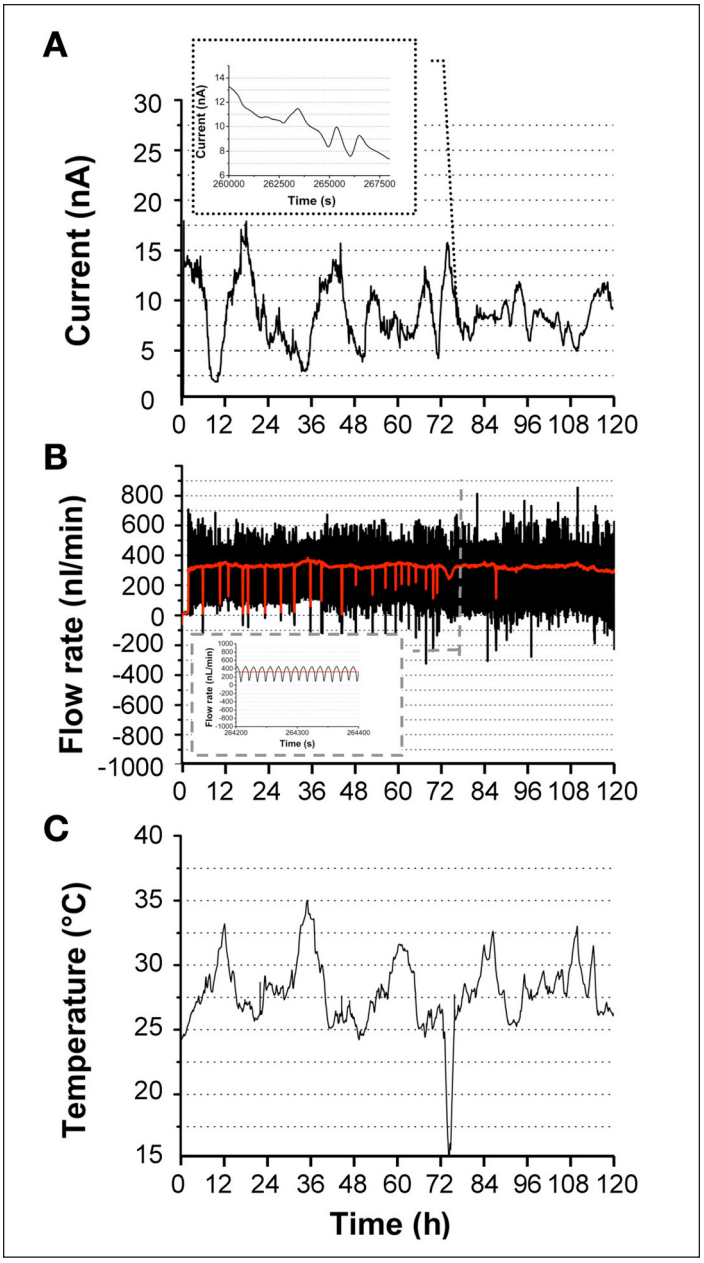

Figure 2. Characteristic *in vivo* data set of a CRT system over a monitoring time of 120 h. **(A)** Exemplary current trace. The insert depicts the achievable signal-to-noise ratio as well as the time resolution of the system. **(B)** Exemplary flow rate (raw data, black; 5 min average, red). As evident from the insert, a highly periodic peristaltic pumping action is recorded by the built-in highly sensitive flow rate sensor (Δt = 1 Hz). Pulse amplitudes vary due to back-pressure fluctuations while having no influence on mean flow rate and glucose sensor signal. Short negative flow rate excursions are caused by bubble elimination in the bubble removal unit. **(C)** Exemplary temperature measured underneath the glucose sensor. Positive temperature peaks coincide with bedtimes, whereas the negative peak after 72 h is due to a foray outside the clinical center.

sensor resistance. The temperature measured underneath the glucose sensor, depicted in **Figure 2C**, was used for temperature compensation prior to calibration of current readouts.

In Vitro System Performance

When bubbles pass the CRT disposable, they are removed from the dialysate flow by continuous application of negative pressure in the bubble filter. Thus the flow of dialysate through the glucose sensor and the flow rate sensor is decreased to zero in the meantime. Consequently, the occurrence of and the time-of-passage of bubbles could be determined by the occurrence of periods during which the mean flow rate was measured to be zero. On average, the flow rate was recorded to be zero during 0.05% of the total monitoring time *in vitro*.

Figure 3A depicts the mean flow rate of 10 systems measured by the built-in flow rate sensors over a total monitoring time of 120 h. The mean of the mean flow rates of the tested systems was measured to be 330 ± 48 nl/min. The mean periodic flow rate variation was 24 ± 7% of the mean flow rate. The relative standard deviation of the mean flow rate was measured to be 14.5%. This deviation is mainly attributed to typical tolerances of 10% and 2% of inner and outer tubing diameters, respectively. Further, manufacturing tolerances of pump tube segments, of the diameter and the excentricity of each roller, as well as of the tube bed contribute to the overall variation of mean flow rate. The recorded periodic variation of mean flow rate ranged from 1% in system 4 to 42% in system 9 (**Figure 3A**). These significant differences of pulsation amplitudes between systems are caused by intrasystem differences of back pressure downstream from the flow-through glucose sensor. Comparably high back pressure from connecting fittings and flow rate sensor capillaries results in high pulsation amplitudes. Periodic variation of the mean flow rate, however, was found to have no effect on current readout due to sufficient dampening of flow pulsation by a cover membrane deposited on top of the working electrode of the flow-through sensor. The sensor sensitivity was found to be independent of mean flow rate changes at flow rates above 250 nl/min. The effect of resulting changes of system lag time onto measurement accuracy was investigated by taking into account a correction factor of mean lag time prior to calibration.

The accuracy, the precision, and the specificity of glucose measurement were determined as follows. The sensor response was determined at exposure to twofold repeated glucose concentration step profiles over a total monitoring time of 120 h at constant

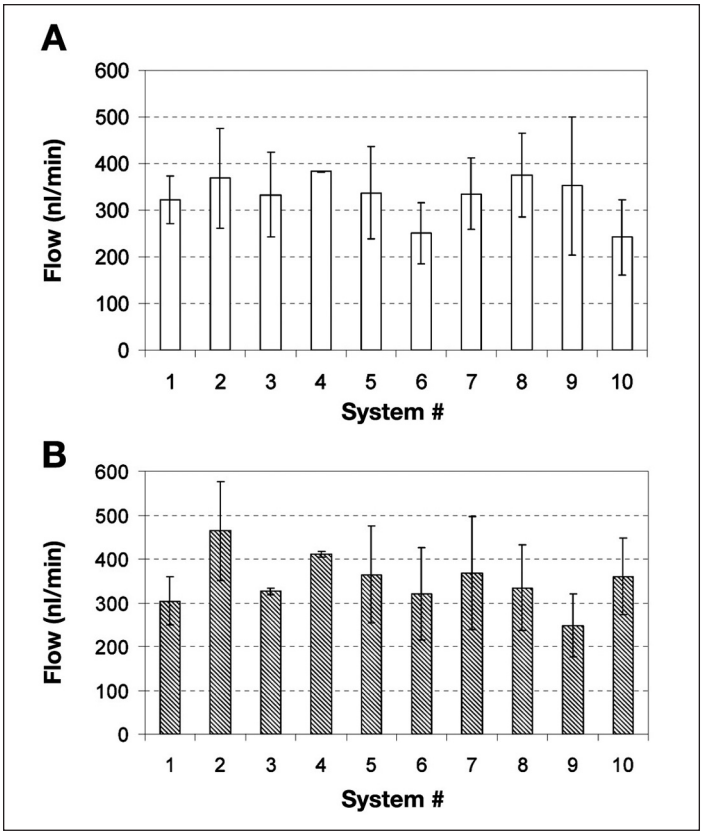

Figure 3. Variation of the mean flow rate of 10 different CRT systems calculated from recorded CRT flow rate sensor measurements over a total monitoring time of 120 h **(A)** *in vitro* and **(B)** *in vivo*.

temperature. Each step profile featured 16 steps of 2 h in length corresponding to 16 different reference solutions (glucose concentration = 0 - 26 x 10^{-3} mol $liter^{-1}$, determined by the Hexokinase method [Hitachi 917, Roche Diagnostics]). Median sensor responses for each reference concentration served to construct a calibration curve for each disposable. Agreement of calibrated and reference glucose concentrations was established by calculating the mean absolute relative error (MARE). Mean MARE (%) values of 10 disposables, randomly sampled out of 6 different batches, are given in **Table 1**. Reproducibility of calibrated glucose concentration during subsequent concentration profiles was calculated for all reference solutions used and is reported in **Table 1** as mean precision value of 10 disposables drawn from 6 different disposable batches. The average of mean MARE and mean precision values characteristic for six disposables batches was determined to be 2.9 ± 0.9% and 2.6 ± 0.8%, respectively. This result points to consistently very good agreement between the established calibration curves and the acquired signals of flow-through sensors. Further, good mean precision suggests very good sensor reproducibility over a monitoring time of 120 h.

The specificity of the glucose readout was characterized by monitoring the sensor response in presence of a selection of potential interferents at physiological concentrations. **Table 2** depicts the change in current readout at a glucose concentration of 10 x 10^{-3} and 20 x 10^{-3} mol liter^{-1}. All substances tested did result in sensor readout changes of less than 10% and may well be considered as insignificant in the euglycemic and hyperglycemic range. Uric acid and gentisic acid are consistently causing the largest signal deviation pointing to electrochemical interferences that may become significant in the hypoglycemic range.

Table 1.
Mean Absolute Relative Errors and Precision of *In Vitro* Measurements

Disposable batch	MARE[a] (%)	Precision[a] (%)
1	3.1 ± 0.8	3.9 ± 0.8
2	3.7 ± 1.9	2.8 ± 2.0
3	2.0 ± 0.6	1.6 ± 0.2
4	2.2 ± 0.7	2.2 ± 0.7
5	2.1 ± 1.6	3.5 ± 6.3
6	4.3 ± 1.4	2.0 ± 0.7
mean	2.9 ± 0.9	2.6 ± 0.8

[a] Ten CRT disposables were measured per batch; monitoring time = 120 h; glucose concentration of reference solutions was 0, 2, 4, 5, 7, 9, 10, 12, 14, 15, 17, 19, 20, 22, 24, and 26 x 10^{-3} mol liter^{-1}; and the buffer was 8 x 10^{-2} mol liter^{-1} 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, pH = 7.4.

Table 2.
Effect of Different Interferents onto the Glucose-Dependent Current Response

Interferent	mol liter^{-1}	Current change[a] (glucose) (10 x 10^{-3} mol liter^{-1})	Current change[a] (glucose) (20 x 10^{-3} mol liter^{-1})
Acetylsalicylic acid	5.5 x 10^{-3}	-4%	-2%
Acetylcysteine	9.2 x 10^{-1}	3%	1%
Ethanol	7.6 x 10^{-2}	-1%	0%
K-oxalate	5.4 x 10^{-2}	5%	4%
Cefoxitin	2.3 x 10^{-3}	2%	2%
Uric acid	4.2 x 10^{-4}	-7%	7%
Dopamine	6.0 x 10^{-6}	2%	2%
Gentisic acid	1.2 x 10^{-4}	6%	4%

[a] Changes of mean current readouts (monitoring time = 120 h; *n* = 8 CRT disposables; glucose concentration = 10 and 20 x 10^{-3} mol liter^{-1}; buffer = 8 x 10^{-2} mol liter^{-1} 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, pH= 7.4) are given for eight different interferent solutions at physiologically relevant concentrations.

In Vivo System Performance

On average, the time period during which the flow rate was measured to be zero was recorded during 0.2% of the total monitoring time *in vivo*, with an overall mean and maximum passage time of bubbles of 3.2 ± 3.2 and 10 min, respectively. The choice of microdialysis catheter was found to be crucial to avoid obstruction of the microdialysis catheter, tube clogging, and thus a drop in flow rate over time. Microdialysis catheters of a nominal cutoff of less than 20 kDa were investigated *in vitro* in serum samples over a monitoring time of 120 h, yielding strongly decreasing flow rate over a total monitoring time of 120 h. In precedent *in vivo* studies, it was further found that the use of microdialysis catheters with polyarylethersulphone (PAES) membranes of a nominal cutoff of 100 kDa and a mean H_2O permeability of 5.3 ± 0.5 x 10^{-3} cm/bar/s resulted in flow rate drops of no less than 10% at the fourth day after insertion. In contrast, no negative flow rate drift was measurable when using microdialysis catheters with PAES membranes with a nominal cutoff of 20 kDa and a H_2O permeability of 5 - 8 x 10^{-4} cm/bar/s, as utilized in this study. **Figure 3B** depicts mean flow rates measured in 10 *in vivo* experiments. The mean of average flow rates of all systems was measured to be 350 ± 59 nl/min, while periodic flow rate variations were measured to amount to 22 ± 6% of the mean flow rate on average of all systems.

Mean positive, negative, and overall rates of change of glucose concentrations were calculated for each CRT system for each experiment. The positive and negative mean of these values (mean of the mean) was 1.17 ± 0.29 and -1.01 ± 0.27 mg/dl/min, respectively. The mean of the respective standard deviations was 2.64 and 2.41 mg/dl/min. The overall mean of these values (mean of the mean) was 0.00 ± 0.01 mg/dl/min. The mean of the standard deviations was 2.82 mg/dl/min.

The performance of the CRT was assessed by calculating the MARE value and the prediction error sum of squares (PRESS) error grid analysis (EGA) plots and plots of calibrated CRT signals over time in comparison with BG readings. Retrospective calibrations of current readouts were performed as follows. Primary sensor signals—current levels (nA)—were processed by eliminating a run-in time of 24 h to rule out artifacts induced by sensor run-in and wound healing. Subsequently, sensor signals were corrected for temperature variations during the experiment and for the time delay between catheter sampling time and detection time at the electrochemical flow-through sensor, the physical lag time. Temperature

compensation was based on the temperature readouts recorded and the compensation factor of 4.5%/°C established *in vitro*, prior to *in vivo* studies. The correction for physical lag time was conducted in two different ways to investigate the effect of interexperiment mean flow rate variations onto system performance. In calibration scheme I, the average physical lag time was determined *in vitro* at a mean flow rate of 330 nl/min by measuring the average response time to reach 95% of the final current level after the induction of a glucose concentration step of 5×10^{-3} mol liter^{-1}. The determined lag time of 33 min was then utilized to correlate BG reference values and current readouts. In calibration scheme II, the physical lag time was compensated for the deviation of mean flow rate recorded in each *in vivo* CRT experiment and the mean flow rate determined *in vitro*.

The signal was then smoothed with a Savitzky–Golay filter (t = 5 min, second degree) to further reduce noise in a frequency range outside feasible time constants of glucose variations, and the system time of reference and continuous monitoring system were synchronized. One calibration curve was established for each CRT experiment. After removal of outliers, capillary blood measurements served to establish two independent calibrations in order to allow for assessment of the effect of calibration frequency onto accuracy: first, all BG values were taken into account for construction of the fit calibration curve, and second, only BG values separated by a time interval of at least 8 h served to construct the calibration curve.

Table 3 contains the mean MARE and PRESS values for calibration schemes I and II. As evident from **Table 3**, the differences of determined mean and median MARE and PRESS values for differently acquired physical lag times do not seem to indicate that an individual physical lag time correction is necessary for this data set. This may be due to insignificant deviation of mean flow rate determined *in vivo* and *in vitro*. The mean physical lag time amounts to 31.9 ± 5.6 min based on individually acquired flow rates in contrast to 33 ± 4.8 min based on *in vitro* measurements described earlier.

The agreement of reference values and continuously monitored glucose values as analyzed by a Clarke EGA is given in **Figure 4** for a different number of reference values used for calibration. **Figures 4A** and **4B** depict the results, taking all BG reference values and all reference values separated by a time interval of at least 8 h into account, respectively. As evident from **Table 3** and from **Figures 4A** and **4B**, the consideration of all reference values does not significantly affect the key figures shown to demonstrate system accuracy, indicating that a smaller number of BG measurements may be required in further studies. Consistent with the MARE and PRESS values given in **Table 3**, the EGAs in **Figures 4A** and **4B** confirm a very good measurement quality of the CRT: 98.0% and 97.6% of all measurements were within zones A and B, respectively. In the range of 70–180 mg/dl, 99.7% and 99.8% of all measurements were in the zones A and B, respectively.

Figure 5 shows exemplary results of two CRT systems applied to each patient. Reference values within the first 24 h are omitted from calibration to eliminate any possible effects of tissue hypoxia, microdialysis catheter

Table 3.
Mean Absolute Relative Errors and Prediction Error Sum of Squares for Two Calibration Schemes

Reference values[a]		MARE (%) mean (n = 10)	MARE (%) median (n = 10)	PRESS (%) mean (n = 10)	PRESS (%) median (n = 10)
All BG values	Calibration scheme I[b]	12.2 ± 5.9	9.6	15.1 ± 6.4	13.6
All BG values	Calibration scheme II[c]	12.3 ± 5.7	11.0	14.6 ± 5.6	13.6
All BG values	ΔScheme I–scheme II	-0.1 ± 8.2	-1.4	0.5 ± 8.5	-0.1
Δt > 8 h	Calibration scheme I[b]	12.4 ± 5.7	10.2	15.5 ± 6.7	14.0
Δt > 8 h	Calibration scheme II[c]	12.7 ± 5.5	12.0	15.2 ± 5.5	14.1
Δt > 8 h	ΔScheme I–scheme II	-0.3 ± 7.9	-1.8	0.3 ± 8.6	-0.1

[a] Run-in = 24 h and monitoring time = 120 h.
[b] Calibration scheme I is based on the mean flow rate of $\bar{\dot{v}}$ = 330 ± 48 nl/min determined *in vitro* and an average lag time of t_{av} 1980 ± 288 s determined *in vitro*.
[c] Calibration scheme II is based on the measured mean flow rate in each experiment $\dot{v}_n$ and a corrected lag time $t_n = t_{av}\frac{\bar{\dot{v}}}{\dot{v}_n}$

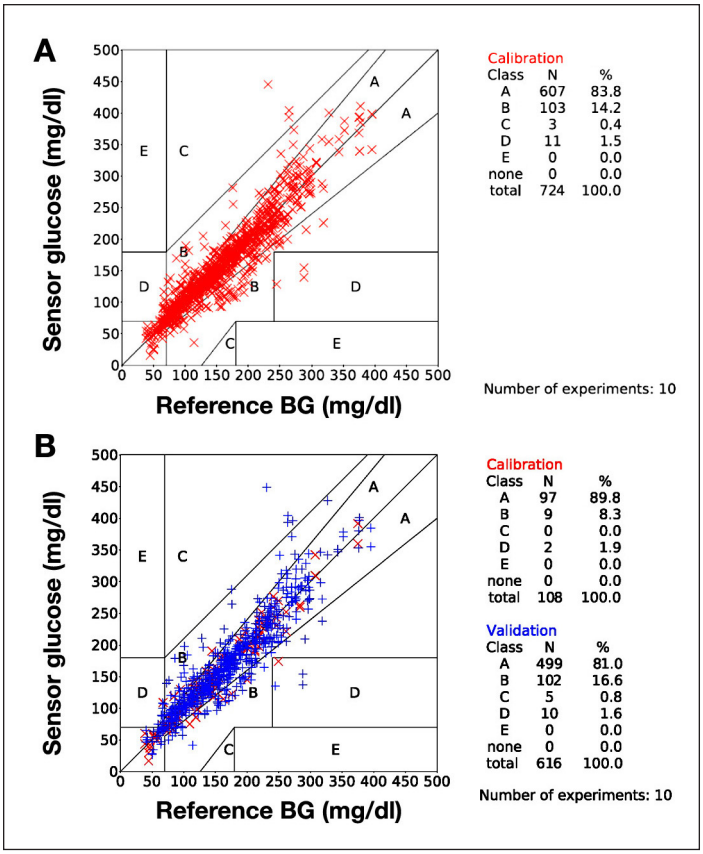

Figure 4. Error grid analysis for retrospective calibration. **(A)** Omitting blood glucose reference measurements within 24 h after system start-up, all reference measurements are used for retrospective calibration. **(B)** Omitting blood glucose reference measurements within 24 h, all blood glucose reference values separated by at least 8 h. Calibration points (3/day) and validation points (>10/day) are depicted red and blue, respectively.

wet-up, and sensor run-in onto calibration. Continuously recorded glucose concentration resulting from calibrated sensor signals is depicted as a solid curve. Qualitatively good agreement between the two systems was found. For this data set and all BG reference values used for calibration, the median absolute and the mean absolute deviations between two CRT systems were 24.2 and 30.2 ± 24.2 mg/dl, respectively (**Table 3**, all BG values, calibration I). For the reduced set of calibration parameters (**Table 3**, $\Delta t > 8$ h, calibration I), the median and the mean absolute deviations were 24.7 and 32.1 ± 27.1 mg/dl, respectively. The median deviations for all five data sets ranged from 9.2 to 30.2 mg/dl, and the overall mean deviation was 27.1 mg/dl with a pooled standard deviation of 32.0 mg/dl when all calibration parameters were used. For the reduced calibration set, the values were 9.6 to 32.6 and 28.5 mg/dl with a pooled standard deviation of 33.4 mg/dl. Glucose concentration, which is depicted as a dashed curve for the first 24 h of monitoring, results from retrospective calibration when using subsequent calibration points, depicted as red circles. Signal artifacts such as the zero signals within the first 12 h of one signal trace, depicted in blue, occurred in 8 of 10 experiments and result from significantly reduced sensor sensitivity during this time period. In all systems, mean flow rate was nearly constant 2 h after system start-up. However, the recorded data set does not allow a distinction between possible physiologically induced temporary decrease of glucose concentration within the vicinity of the catheter and prolonged run-in time of the glucose flow-through sensor.

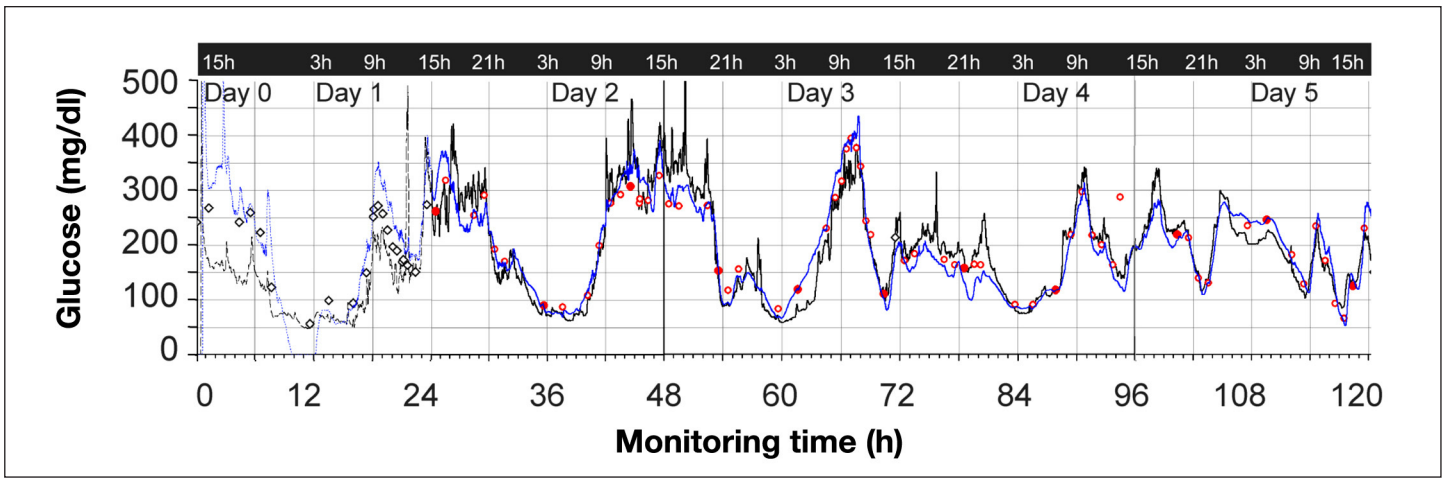

Figure 5. Superimposed exemplary data of one subject with two independent CRT systems (black, blue). Blood glucose values, used to construct the calibration curve, are depicted as filled red circles (●). Blood glucose values during a run-in time are omitted from the calibration and depicted as open diamonds (◇), while BG values used for validation are given as open red circles (○). Glucose concentration values that are calibrated using the calibration points (●) are depicted as dashed and continuous curves for the time period prior to and after 24 h of monitoring, respectively. Catheters were symmetrically implanted in the periumbilical area. Signals are calibrated according to calibration scheme I, with a set minimum time interval of 8 h between calibration points.

Conclusions

The first *in vivo* investigations with the new microdialysis tool presented show that very good measurement accuracy over a total monitoring time of 120 h can be achieved when several intrinsic pitfalls of any microfluidic system are avoided. Primarily, the flow rate constancy over time, the constancy of glucose recovery, and the elimination of bubbles prior to entry in a flow-through sensor have proven to be crucial to accurate measurement and reliable performance. This was mainly achieved by implementing a rugged peristaltic pump and a simple yet very efficient bubble removal unit. The main effort to further reduce measurement error will now be directed toward further improvement of sensor characteristics.

The multitude of implemented system controls and recorded system parameters is very useful in validating the quality of each *in vivo* experiment. For example, any accidental removal of the catheter or unintentional damage to the tubing or catheter can be detected by the flow rate alarm, allowing the health care professional to intervene. In addition, the passage of a bubble through the catheter and the tubing downstream can be detected by a decreased flow rate and increased sensor current and counter electrode voltage. This allows for artifact detection. Furthermore, the flow rate information will be extremely valuable to explore the possible maximum monitoring time of the CRT, which is fundamentally limited by catheter clogging. During the monitoring time of 120 h set for this study, no decrease in mean flow rate was detected. The system is currently designed for use up to 7 days, limited by the volume of the perfusion liquid stored in the reservoir bag. Further investigations will be needed to study the maximum possible time of flow rate constancy for the given perfusion liquid, catheter membrane, and insertion mode.

Clearly a reduction of run-in time would be of interest to the user. In the presented experiments, we observed that sensor and microdialysis catheter wet-up, as monitored by sensor current and flow rate, respectively, were not limiting the reduction of system run-in time. Based on the recorded data sets, we assume that system run-in time can be reduced to significantly less than 24 h. Further studies will be needed to investigate the cause and the mean time period of decreased sensor sensitivity after system start-up.

Needle-type sensing systems offer shorter response time and simpler system concepts than microdialysis-based systems such as the CRT. The CRT has a lag time of 33 min, has to be calibrated retrospectively, and thus prevents use as a real-time system. Advantageously, however, due to the relative simplicity of an *ex vivo* sensor, the CRT does not have to be recalibrated during monitoring. All results presented are based on one calibration per experiment. The CRT is a modular system. In this study, a specific biocompatible microdialysis catheter, isotonic saline as perfusion liquid, and a second-generation glucose flow-through sensor were used. Further improvements in any of the components are easy to implement and will lead to further improvements of system performance. Most importantly, the CRT proved to be a very robust and reliable system. In contrast to other known microdialysis systems, the CRT displays and records all relevant system parameters. It consequently allows differentiation between artifacts and true physiological events, a key requirement for deriving meaningful results from clinical studies, and further improvement of system performance.

In summary, the CRT is an accurate continuous glucose system based on microdialysis. It is an attractive, robust tool for measuring glucose concentration in interstitial fluids for a minimum period of 120 h. The CRT will thus be very useful as a reference system.

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