CONTINUOUS BLOOD GLUCOSE MONITORING USING MICRODIALYSIS

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DOCTORAL THESIS

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Preface and statutory declaration

This work was carried out from January 2005 – April 2013. It summarizes four peer-reviewed publications and a book chapter (Franz Feichtner et al. 2010; Franz Feichtner et al. 2011; H. M. Heise et al. 2008; Heise et al. 2010; Julia K Mader et al. 2012) which I published as first- and co-author. In addition, several previously unpublished technical and experimental details are presented. For easier readability and to guide the reader through the development process, I decided not to just reprint my already published first-authorship publications but rather decided to arrange the content in a way such that published and unpublished content present a new "big picture".

Therefore, some chapters or sub-chapters are representations of the original manuscripts or parts of the original manuscripts that have been adapted. I have marked the relevant passages at the beginning of each chapter.

STATUTORY DECLARATION

I declare that I have authored this thesis independently, that I have not used other than the declared sources / resources, and that I have explicitly marked all material which has been quoted either literally or by content from the used sources.

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From here to there to every place in between!

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Abstract:

Driven by the reported medical benefit of (tight) glycaemic control of ICU patients the EC funded project CLINICIP was initialized. CLINICIP aimed to develop a system for closed loop insulin infusion in critically ill patients. This thesis is embedded in CLINICIP and focussed on the development, the technical and clinical evaluation of an extravascular microdialysis based body interface for continuous glucose monitoring. Several approaches were investigated technically and in a risk assessment study in order to find an appropriate design and optimal operating conditions. The final design of the developed body interface features a system for continuous blood withdrawal and a planar flow-through microdialyser that delivers a protein-free blood dialysate. Following a stepwise approach, this body interface was further combined with spectrometric and amperometric online glucose sensors and later on with an algorithm to function as a continuous glucose monitoring and regulation system. The technical and clinical performance evaluations of this system and its subsystems were done in clinical studies in healthy volunteers and type 1 diabetes mellitus patients. Results from these investigations using the extravascular microdialysis approach were published peer-reviewed as first author. The results of a comparison to similar tests using subcutaneous microdialysis probes were peer-reviewed published as co-author.

Key words:

CLINICIP, closed loop systems, extravascular microdialysis, sampling, glucose monitoring, glucose sensing, metabolic control, algorithms, model predictive control, in vitro, in vivo, clinical trial, intensive insulin therapy, ICU

ABBREVIATIONS

ADA	American Diabetes Association
AP	Artificial Pancreas
APTT	Activated partial thromboplastin time (measure for a patient's coagulation status)
APACHE II	Acute Physiology and Chronic Health Evaluation II; a severity-of-disease classification system
BG	Blood glucose
CGM	Continuous Glucose Monitoring
CLINICIP	Closed Loop Insulin Infusion for Critically Ill Patients
DIA	Dialysate samples
ECF	extracellular fluid
GCP	Good Clinical Practice
ICU	Intensive care unit
IFG	Interstitial fluid glucose
IIT	Intensive insulin treatment
ISO	International Standard Organisation
IV	intravenous, intravascular
MD	Microdialysis
MPC	Model predictive control
OGTT	Oral glucose tolerance test
PFTMD	Planar flow through microdialyser
RCT	Randomized controlled trial
REF	Reference blood samples
Sc	subcutaneous
T1DM	Type 1 diabetes mellitus
TGC	Tight glycaemic control

CONTENTS

CHAPTER I

Introduction – setting the scene

1. Tight glycaemic control in critically ill patients

Intensive care patients are reportedly threatened with stress-induced diabetic symptoms like hyperglycaemia and insulin resistance (Mizock 1995; McCowen et al. 2001). These symptoms are associated with adverse clinical outcome such as myocardial infarction, polyneuropathy and multiorgan failure (Malmberg et al. 1999; G van den Berghe et al. 2001; G. E. Umpierrez et al. 2002). In fact, stress-induced hyperglycaemia is associated with an increased risk of death in patients with and without diabetes (Capes et al. 2000).

In a izlandmark study by Greet van den Berghe in 2001, 1548 mechanically ventilated surgical ICU patients were enrolled in a single centre randomised controlled trial to investigate whether normalisation of blood glucose levels with intensive insulin therapy reduces mortality and morbidity among critically ill patients. The study showed that strict glycaemic control led to a significant reduction of mortality and morbidity among diabetic and non-diabetic ICU patients and reduced the length of ICU-stay (Greet van den Berghe et al. 2001). Mortality was reduced from 8.0% in the conventional treatment group (mean morning BG level 8.5±1.8 mmol/l) to 4.6% in the group receiving intensive insulin therapy (mean morning BG level of 5.7±1.1 mmol/l). The target glucose range was set between 4.4 and 6.1 mmol/l (=80-110mg/dl) and was adjusted based on a 1-4 h BG measurement interval. The algorithm was later presented in detail (G Van den Berghe 2002; Greet Van den Berghe et al. 2003) and became well-known as the “Leuven insulin titration guideline”.

Also patients admitted to medical ICUs for at least three days were reported to benefit from tight glycaemic control (TGC). Intensive insulin therapy (IIT) decreased morbidity but did not reduce in-hospital mortality (Greet Van den Berghe, Wilmer, et al. 2006).

In children an improvement of the short-term outcome for patients in the paediatric ICU was determined by Vlasselaers and co-workers (Dirk Vlasselaers et al. 2009). Others could only find a small effect (Beardsall et al. 2008) and some found no effect at all (M. S. D. Agus et al. 2012).

Krinsley also reports a significant reduction of mortality (- 29.3%), organ dysfunction and length of stay in a heterogeneous population of 800 critically ill adult patients in a medical-surgical ICU (J. S. Krinsley 2004). He found that the reduction of mortality was directly proportional to the patients’ APACHE-II-score and was best for patients with an APACHE-II score below 15 (-73.8%). However, Krinsely used a different insulin titration protocol compared to Greet van den Berghe and he tried to maintain glucose at a higher upper concentration of 7.7 mmol/l. This level was

achievable without increasing the risk of treatment-related severe hypoglycaemia (J. S. Krinsley 2004).

Follow-up studies from Leuven (Hermans et al. 2007; Langouche et al. 2005), other ICU teams (Grey & Perdrizet 2004; Gabbanelli et al. 2005; Finney et al. 2003; J. S. Krinsley 2004) and reviews (Thomas et al. 2007) also supported the first van den Berghe findings, which – in the meantime – have found their way into clinical ICU-practice (Bagshaw, Egi, et al. 2009; Bagshaw, Bellomo, et al. 2009; Baldwin et al. 2005; Aragon 2006). In a large multicentered study including >66,000 patients in 24 Australian ICUs, Bagshaw and co-workers found that mean BG values decreased for mechanically ventilated surgical and cardiac surgical patients. Interestingly those data showed an important association of both low and high BG values with hospital mortality (Bagshaw, Egi, et al. 2009) and also a correlation between early BG variability (incidence of both hypo- and hyperglycaemia in the first 24 hours) and an increased risk of mortality (Bagshaw, Bellomo, et al. 2009).

These favourable findings have prompted health care organisations to incorporate tight glycaemic control into ICU guidelines, among others the American Association of Clinical Endocrinologists (Moghissi et al. 2009), the Surviving sepsis campaign (Dellinger et al. 2008) and the American Diabetes Association ADA (American Diabetes Association 2008).

On the other hand, several multicentre studies failed to confirm the Leuven findings:

- **GIST-UK trial:** the GIST-UK trial failed to reproduce a reduction in mortality while managing post-stroke hyperglycaemia of >900 patients in the UK. The trial was stopped early due to slow enrolment. However, their findings suggest that significantly reduced plasma glucose concentrations cannot be associated with a significant clinical benefit (Gray et al. 2007).
- **VISEP trial:** In their attempt to investigate the effect of IIT on mortality and morbidity of patients with severe sepsis, the study team stopped the trial early for safety reasons after having enrolled >500 patients. The mean BG of the intensive-therapy group was lower than in the conventional group. However, the reduced BG level led to a significant increase of hypoglycaemic events and increased hypoglycaemia-related serious adverse events (Brunkhorst et al. 2008).
- **GLUCONTROL study**: In a trans-European randomised controlled multi-centre study medico-surgical ICU-patients were randomised to a tight glycaemic control group (target

BG: 4.4-6.1mmol/l) or to a moderate glycaemic control group (target BG 7.8–10.0 mmol/L). The trial was also stopped early for safety reasons, since there were too many unintended protocol violations. The clinical benefit was not evident for the TGC-group. Moreover, this group experienced an increased incidence of hypoglycaemic events compared to the moderate glycaemic control group (Preiser et al. 2009).

- **NICE-SUGAR:** The largest, international, randomised controlled trial aimed to find the optimal target range for blood glucose in critically ill patients to reduce 90 day mortality. More than 6100 patients were randomly assigned to a tight glycaemic control group (4.5 to 6.0 mmol/l) or to a conventional glucose control group with a glycaemic target range of <10.0 mmol/l. The NICE-SUGAR study investigators found that tight glycaemic control – in direct contrast to the Leuven-findings – increased the absolute risk of death compared to the conventional group at 90 days by 2.6 percentage points (Finfer et al. 2009) – compare **Figure 1**. In conclusion the study investigators did not recommend to use the lower target in critically ill adults (Finfer et al. 2009).

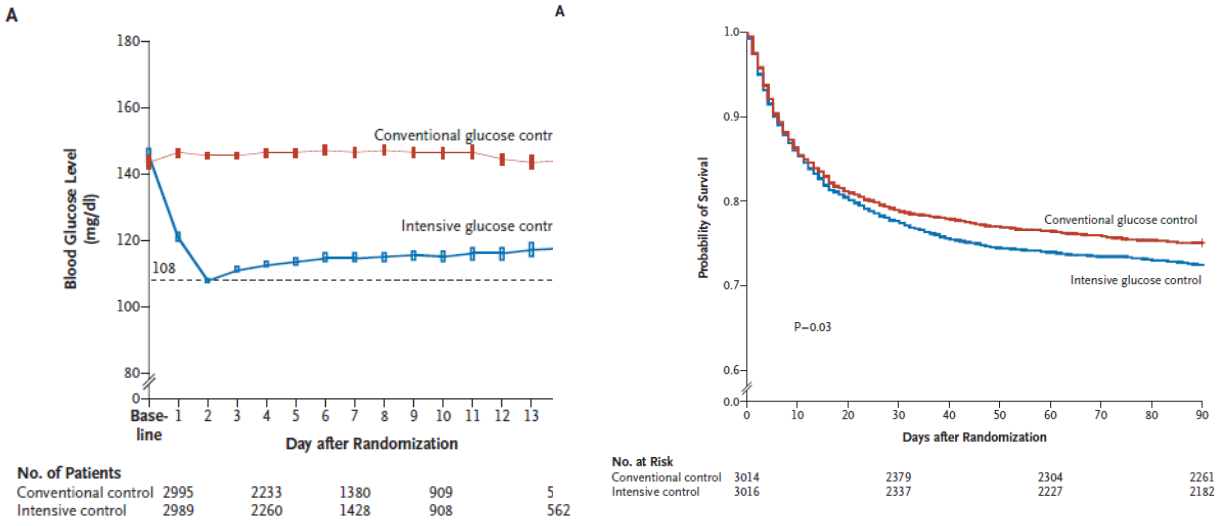


Figure 1: Results of the NICE-SUGAR study showing that intensive glucose control compared to conventional glucose control (left) leads to a decreased survival probability (right); (Finfer et al. 2009).

2. From tight to safe glycaemic control in critically ill patients

In two meta-analysis (R. S. Wiener et al. 2008; Griesdale et al. 2009) glucose control-studies were tested for a common potential beneficial or detrimental effect of tight or moderate glucose control in adult intensive care patients. In addition, Kansagara and co-workers reviewed IIT-

studies and evaluated risks and harms in order to guide recommendations of the American College of Physicians for the management of inpatient hyperglycaemia (Kansagara et al. 2011).

All three analyses revealed that TGC in ICU patients is not associated with a general reduced mortality or with a general reduced length of hospital stay. However, Griesdale et al. and Wiener et al. found that the subgroup of surgical ICU adults benefitted from IIT, while the risk of severe hypoglycaemia increased 5- to 6-fold, respectively (Griesdale et al. 2009; R. S. Wiener et al. 2008). The authors of the meta-analysis hypothesised that protocol differences might have contributed to the fact that many studies were not able to replicate the Leuven results. The positive IIT-effects from Leuven might depend on one or more of the following factors that differed between the first Leuven study (Greet van den Berghe et al. 2001) and other study protocols. In summary the following factors might have contributed to the reported different study outcome, either individually or in combination (Griesdale et al. 2009; R. S. Wiener et al. 2008; Greet Van den Berghe et al. 2009; Sacks 2009) – Leuven study settings are underlined:

- **ICU setting:** surgical ICU vs. medical or mixed ICUs
- **Insulin infusion route:** central venous lines for continuous infusion vs. peripheral venous or subcutaneous (including bolus injections)
- **Blood sample origin:** arterial vs. capillary, venous or arterial blood samples
- **Glucose meters:** highly accurate arterial blood gas analyser vs. inaccurate point-of-care devices or laboratory analysers
- **Blood matrix:** whole-blood vs. plasma
- **Nutrition:** parenteral vs. enteral
- **Target ranges:** 80-110 mg/dl vs. other ranges

Success might also depend on staff experience and on the ability of the nursing staff to maintain blood glucose in the defined target range (Greet Van den Berghe et al. 2009).

In summary, the debate over tight glycaemic control in ICUs is not over yet, but it moves towards a less generalised but more patient and situation-oriented one. One consensus might be a re-definition of the target glucose range. The term “tight glycaemic control” might shift towards “safe glycaemic control” – i.e. a higher, moderate target glucose range, e.g. 7.8-11.1 mmol/l (Kansagara et al. 2011) or 7.7 – 10 mmol/l (Preiser & Devos 2007), which is recommended as the

target level with the lowest risk-to-benefit ratio – independent of the patient population (Preiser & Devos 2007).

In the meantime, the Society of Critical Care Medicine published a guideline (Jacobi et al. 2012) on glycaemic control in general intensive care unit patients, perioperative patients, postoperative cardiac surgery patients, post-traumatic injury patients, and neurologic injury patients. It suggests glycaemic control end points such that a blood glucose ≥150 mg/dl (=8.3 mmol/l) triggers interventions to maintain blood glucose below that level and at an absolute level <180 mg/dl (=10mmol/l).

3. Technical implementation of Intensive Insulin Therapy in ICUs

The Leuven study (Greet van den Berghe et al. 2001) initialised a worldwide debate on glucose monitoring in ICUs. Glucose control made its way to the ICU wards, but physicians and nursing staff are looking for more or less automated systems to technically implement, standardise and optimise IIT in the ICU. In a multicentre survey 83% of the interviewed nurses and physicians believed that greater emphasis on optimal glucose control is necessary (McMullin et al. 2004). In terms of improving current strategies, clinicians called for more effective insulin delivery and glucose monitoring.

1) TGC is a major additional workload for the nurses

The working steps that must be performed by the nursing staff include periodical blood sampling, transfer of the blood samples to a blood glucose analyser, calculation of the insulin titration dose according to the protocol, calculation of the amount of infused glucose to provide information for the calculation of further insulin doses, insulin application, alarming, and documentation. This additional workload to establish and maintain TGC in critically ill patients was found to average at 4.72+/-1.13 minutes per BG measurement and was estimated to add up to 2 hours per patient per 24 hours for hourly BG monitoring (Aragon 2006). Nurses call for easier clinical methods for glucose monitoring using arterial catheters to obtain blood samples instead of excessive capillary blood sampling using finger pricks (Aragon 2006).

2) TGC is burdensome and associated with a higher risk of hypoglycaemia

The unconscious or even conscious fear of hypoglycaemia may influence a nurse's or physician's decision on an insulin infusion rate, which in turn influences the quality of TGC. A Canadian multicentre survey (McMullin et al. 2004) showed that nurses had a significantly

higher perceived threshold for hyperglycaemia in non-diabetic patients relative to physicians, probably also reflecting the fear of hypoglycaemia.

3) **TGC requires frequent and accurate glucose measurement**

4) **TGC requires frequent and accurate insulin administration**

5) **TGC is cost effective:**

In a post-hoc cost analysis of the first Leuven study, van den Berghe found intensive insulin treatment to be even more cost-effective than conventional treatment (approx. EUR 2,600), despite higher nursing workload at the ward – as a result of reduced morbidity and length of in-hospital stay (Greet Van den Berghe, P. J. Wouters, et al. 2006).

Ng and Curley conclude aptly that "research on anchoring and supportive techniques will provide an evidence-based approach to future clinical protocol implementation. This is important because ICU nurses need fewer things to think about." (Ng & Curley 2012)

3.1 CLINICIP Project

After the first "TGC in ICU" publications an EC funded project was initialised – CLINICIP (Closed loop insulin infusion for critically ill patients; Project Reference: IST FP6-506965). It aimed to develop an intelligent, low-risk glucose monitoring and control system to help ICU staff implementing glucose control in the critically ill. The overall goal of this international project was to establish glycaemic control in order to improve survival chances in ICUs and increase efficiency and safety in clinical practice (The CLINICIP consortium 2008).

The concentration of vital parameters (e.g. glucose, lactate, O_2, CO_2,…) can be monitored by frequently taking arterial or venous blood samples. However, blood consumption is crucial especially in this patient group, taking blood samples is time consuming and labour-intensive. What's more, the results of these measurements need to be readily available to allow adequate decision making and ensure adequate therapy. Thus, a fast, low-volume, precise on-site monitoring and control system is required.

A schematic block diagram of the CLINICIP main components is shown in **Figure 2**. The very first part – and also one of the most critical parts – of a glycaemic control system is the automatic and continuous glucose monitoring system. It includes a glucose sensor, which – for biocompatibility reasons – is located outside the body, and a "body interface" that delivers a biofluid from the patient to the glucose sensor. This thesis focuses on the development of the latter one – the body interface which fluidically connects the patient with the online glucose sensor.

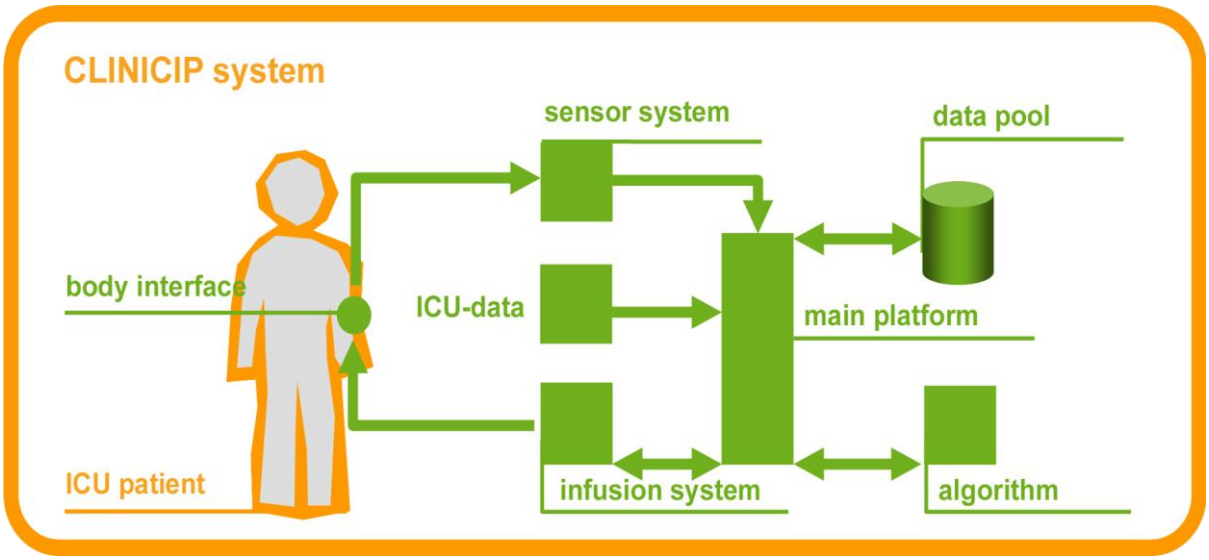


Figure 2: Schematic block diagram of the main CLINICIP components

3.2 Objectives

The objectives of this thesis were to

1) **design, develop and preclinically evaluate a body interface for continuous blood glucose monitoring**.

   The findings are published as peer reviewed publications and are patented. Chapter II summarises these two publications and presents additional so far unpublished data.

   - **Feichtner F**, Schaller R, Fercher A, et al. Microdialysis based device for continuous extravascular monitoring of blood glucose. *Biomedical Microdevices*. 2010;12(3):399-407.
   - **Feichtner F**, Schaupp L, Köhler H. Devices for and methods of monitoring a parameter of a fluidic sample by microdialysis. EP1962993-B1

2) **technically and clinically evaluate the extravascular microdialysis based body interface for continuous blood glucose monitoring in a clinical study.**

   The results are published as peer reviewed publication and are presented in chapter III together with additional so far unpublished data:

   - **Feichtner F**, Schaller R, Fercher A, et al. Microdialysis based device for continuous extravascular monitoring of blood glucose. *Biomedical Microdevices*. 2010;12(3):399-407.

3) **compare the performance results of the developed body interface approach to those obtained with standard subcutaneous microdialysis in a post-hoc analysis of two clinical studies.**

   The results are presented in chapter IV as reprints of the study findings that were originally published as a peer reviewed publication:

   - Mader JK, **Feichtner F**, Bock G, et al. Microdialysis-A versatile technology to perform metabolic monitoring in diabetes and critically ill patients. *Diabetes Research and Clinical Practice*. 2012;97:112-118.

4) **integrate online glucose sensors to form a continuous glucose monitoring (CGM) system that is technically and clinically evaluated in a clinical study.**

   Results are presented in chapter V.

5) **compare the performance results of the developed CGM system to a CGM system based on subcutaneous microdialysis in a post-hoc analysis of two clinical studies.**

   The results are presented in chapter VI as reprints of the study findings that were originally published as peer reviewed publications and in a book chapter:

   - Heise HM, Kondepati VR, Damm U, Licht M, **Feichtner F**, et al. Microdialysis based monitoring of subcutaneous interstitial and venous blood glucose in Type 1 diabetic subjects by mid-infrared spectrometry for intensive insulin therapy. In: *Proceedings of SPIE*.Vol 6863. Spie; 2008:6863 686308-1.
   - Heise HM, Damm U, Kondepati VR, Mader, JM, Feichtner F, Ellmerer M, 2010. Continuous Blood Glucose Monitoring by Infrared Spectrometry as an Important Tool in Clinical Research and Therapy for Improving Glycaemic Control in Diabetic and Critically Ill Patients. In Z. Drzazga & K. Ślosarek, eds. *Some Aspects of Medical Physics – in vivo and in vitro Studies*. Polish Journal of Environmental Studies.

6) **“close the loop” – to integrate a model predictive control algorithm to form a semi-automatic closed-loop blood glucose control device and to evaluate it in a clinical study.**

   The results are published as a peer reviewed publication and are presented in chapter VII together with additional so far unpublished data:

- **Feichtner F**, Mader JK, Schaller R, et al. A stepwise approach toward closed-loop blood glucose control for intensive care unit patients: results from a feasibility study in type 1 diabetic subjects using vascular microdialysis with infrared spectrometry and a model predictive control algorithm. *Journal of Diabetes Science and Technology*. 2011;5(4):901-5

CHAPTER II

Design, development and preclinical evaluation of a body interface for continuous blood glucose monitoring

This chapter is partly taken from a previously published article (Franz Feichtner et al. 2010) and is complemented by so far unpublished data

1. Interstitial vs. blood CGM systems

Most current CGM systems measure the interstitial fluid (ISF) glucose concentration in the subcutaneous adipose tissue. It is however questionable whether ISF glucose concentration accurately reflects plasma glucose concentration – especially in the critically ill.

Blood and the interstitium have different characteristics and Cengiz & Tamborlane thus suggest to consider them as separate glucose compartments (Cengiz & Tamborlane 2009). Interstitial glucose concentration lags plasma glucose concentration in terms of time and absolute concentration. Physiological time lags may account for a few minutes up to half an hour or more (Regittnig et al. 2003; Kulcu et al. 2003; Lourido et al. 2002; Cengiz & Tamborlane 2009). Moreover, a gradient between plasma and interstitial glucose concentration was reported to be persistent (Cengiz & Tamborlane 2009) and may account for approximately 20% (Sternberg et al. 1996).

It is evident that significant physiological time delays as well as significant gradients are present between interstitial fluid of subcutaneous adipose tissue and blood glucose concentrations, depending on a broad spectrum of individual factors including age, weight, blood tissue perfusion, health status and absolute glucose concentration. Lourido et al. and Vlasselaers et al. report on bad correlation between blood and ISF glucose concentration in critically ill patients with severe traumatic brain injuries (Lourido et al. 2002) and critically ill children (Dirk Vlasselaers et al. 2007), respectively. The interested reader is referred to a recent review by Cengiz and Tamborlane debating ISF and BG correlation (Cengiz & Tamborlane 2009).

It is concluded that **a stable, robust and clinically applicable CGM system for critically ill patients** that allows fast and reliable decision making **cannot be based on interstitial but on plasma measurements**. It is therefore obvious not to access ISF but blood for continuous glucose monitoring in intensive care patients, moreover, because blood access is available in these patients anyway. Commercially available and CE-labelled subcutaneous CGM systems and microdialysis based body interfaces were not considered for the development of an ICU-CGM system, although several studies had already been published at that time using subcutaneous CGM systems or microdialysis based probes as body interfaces in ICU patients (J. De Boer et al. 1994; Hutchinson et al. 1999; Lourido et al. 2002; J Kremen et al. 2006).

2. Microdialysis based body interface[1]

It is known that cell and protein adhesion to the sensor surface forms a diffusion limiting barrier (Ward 2008). This effect is also known as biofouling and results in a decrease of sensor sensitivity. Membranes are commonly used in biosensor applications to filter body fluids before delivering it to the sensing unit. Large molecules like proteins and bacteria are hindered on traversing the membrane and adhering to the sensor surface, thus preventing sensor degradation due to biofouling. Hence, the issue of biocompatibility is transferred from the sensor to the body interface, which allows more flexibility in the choice of the used sensor materials.

Therefore it was decided at the beginning of the thesis that the body interface shall provide a protein free matrix for the online glucose sensor, that itself shall be outside the body (ex-vivo). As a consequence a microdialysis based body interface was implemented.

2.1 Short introduction into microdialysis

Microdialysis (MD) is a catheter-based tissue sampling method that goes back to the work of Ungerstedt (U Ungerstedt 1991). It allows the continuous determination of analyte concentrations of the interstitial fluid in various compartments/tissues of interest (e.g. brain, blood, adipose tissue, dermis,…). MD probes today are commercially available for preclinical and clinical use in several tissues.

A MD probe is inserted into the tissue of interest. A perfusion solution (perfusate) is pumped through one lumen of the MD-probe to a semi-permeable membrane, which (in-situ) separates perfusate and interstitium. While perfusate is pumped alongside the membrane, ISF substances below the membrane's molecular weight cut-off may diffuse to the perfusate. After having passed the membrane, the analyte-enriched perfusion solution is called dialysate. It is further pumped to the MD-probe outlet and can be collected for subsequent chemical analysis to obtain highly time-resolved, continuous information about the unbound concentration profiles of the analyte of interest (compare Figure 3 (Plock & Kloft 2005)). As the extracted samples are protein free, there is no need for post sampling preparation.

[1] Partly taken and adapted from (Franz Feichtner et al. 2006) and (Franz Feichtner et al. 2010) and complemented by so far unpublished data

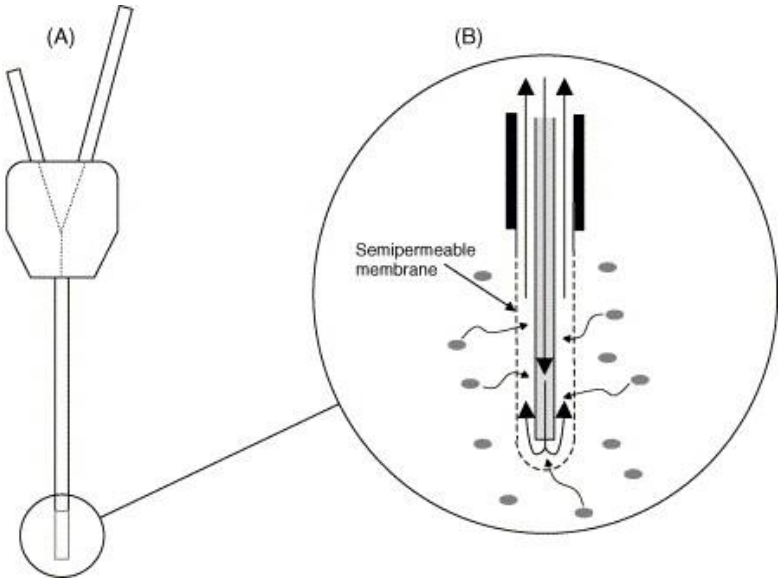


Figure 3 Schematic illustration of a concentric MD-probe with a semipermeable membrane, perfusion in- and outlet (A). (B) shows the probe tip including the semipermeable membrane. Molecules freely diffuse into the probe. Arrows indicate flow direction (Plock & Kloft 2005).

The dialysate concentration never fully equilibrates with its surrounding ISF concentration but it is related to it and depends on parameters such as perfusion flow rates, perfusate and tissue temperatures, the analyte's molecular weight, its charge, osmotic pressure differences and the membrane's exchange surface area.

A measure for equilibration of the dialysate with its surrounding tissue is the so called relative recovery, which can be calculated as the ratio between the dialysate glucose concentration [$Glucose_{DIA}$] and the tissue concentration [$Glucose_{tissue}$]

$$recovery = \frac{[Glucose_{DIA}]}{[Glucose_{tissue}]}$$

Disadvantages of microdialysis:

- probe implantation may elicit tissue reactions which may interfere with the system under investigation
- risk of infection when implanting a MD probe
- lipophilic drugs may stick to tubings and probe components. Therefore it can be assumed that the in vivo recovery of an analyte is not stable over time. Monitoring the relative recovery via certain marker substances (Lukas Schaupp et al. 1999; Yokel et al. 1992) can be advantageous

- molecules larger than the molecular weight cut-off cannot traverse the membrane
- the analyte concentrations in microdialysis samples do not fully equilibrate with the surrounding tissue unless inapplicably low perfusion flow rates (~1 µl/min) are chosen (Ekberg et al. 2005; Rosdahl et al. 1998). Thus, MD data always require calibration procedures

The interested reader is referred to the following reviews on microdialysis that further focus on the theoretical background, MD designs and current applications (Chaurasia et al. 2007; de Lange et al. 2000; Verbeeck 2000; Plock & Kloft 2005).

Conclusion:

As a result of the previously described technical and physiological considerations it was decided that this CGM system will be developed based on blood microdialysis – despite the afore mentioned disadvantages. Two general concepts are possible (intra- and extravascular microdialysis) and will be presented in more detail in the following sections, including a thorough state of the art analysis.

A more general overview of other CGM or artificial pancreas (AP) concepts can be found here: (Klonoff 2007; Skyler 2009; Penfornis et al. 2011). However, none of these AP-systems is commercially available and none of the CGM-systems may be used as decision support system for therapeutic treatment.

2.2 Intravascular microdialysis (iv-MD)

2.2.1 Principles of iv-MD

In iv-MD a microdialysis probe is inserted into a blood vessel via a standard peripheral venous catheter (**Figure 4**). The membrane protrudes from the distal catheter tip and is in direct contact with blood inside the vessel, where the microdialysis process takes place. No blood samples need to be taken for glucose analysis, which makes the technique attractive especially in neonates and children, because blood consumption is extremely crucial in this patient group. Temperature – a parameter of great influence in microdialysis – is constant. On the other hand membrane length and thus membrane exchange surface – another parameter influencing microdialysis recovery performance – is limited in iv-MD.

The probe must be easy to insert, the membrane itself has to be biocompatible of course and must be surface treated to withstand protein and cell adhesion, which was repeatedly reported

to cause decreasing microdialysis efficiencies of membranes exposed to blood (De Lange et al. 2000) in vitro and in preclinical in vivo studies shortly after probe implantation (Yokel et al. 1992; Chen & Steger 1993; Yang et al. 1997; Verbeeck 2000) even with heparinised blood (Sauernheimer et al. 1994).

Furthermore the membrane must be extremely robust, so that friction forces won't result in membrane particle loss, which in turn might have adverse health impacts, like embolism.

Despite these challenges, researches have recognised the high potential of intravascular microdialysis and the numerous thinkable applications in metabolic research associated with the possibility to continuously sample free systemic concentration of metabolites, drug, etc. in vivo (pre-clinically and clinically) without blood consumption.

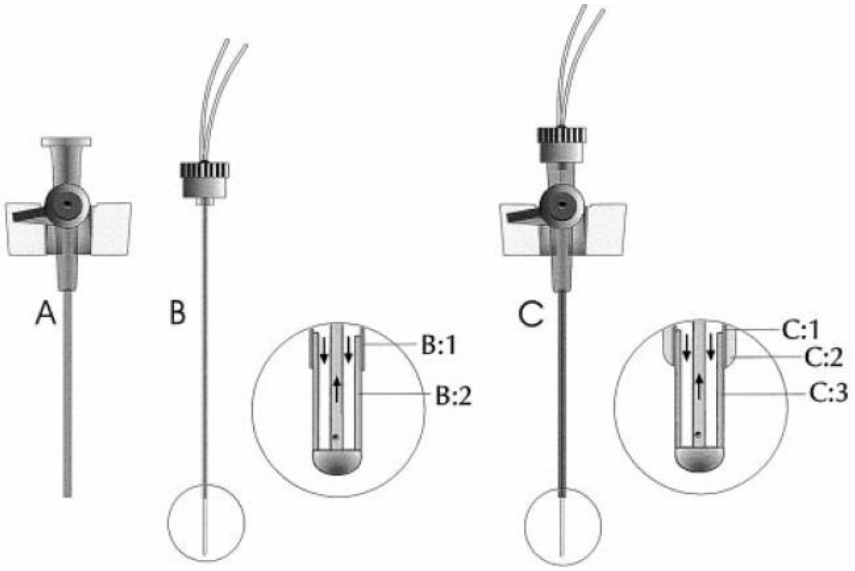


Figure 4: Intravascular microdialysis probe as suggested by Yang et al. (Yang et al. 1997). It can be seen, that the membrane protrudes from the catheter tip and microdialysis can thus be performed directly inside the blood vessel.

2.2.2 iv-MD – State of the art

Years before the van den Berghe study (Greet van den Berghe et al. 2001) initiated the debate about tight glycaemic control of intensive care patients, Stjernstrom and co-workers already measured different metabolites, including glucose, in intensive care patients accessing the patients intravenously. They first used a technique of intravenous microdialysis (Stjernström et al. 1993), but only a few publications have been published since then repeating this technique in humans (Mark T. O'Connell et al. 1996; Patsalos et al. 1996; Páez & Hernández 1997; Castejon et

al. 1999; F. Costa et al. 1999; Nil Dizdar et al. 1999; N. Dizdar et al. 1999; Elshoff & Läer 2005). All of these probes were hand- or custom-made.

Commercial iv-MD probes were not available during the course of the CLINICIP project. A reason for that might be the risks associated with implanting a fragile membrane into the vascular system. If the membrane breaks inside the vessel as a result of mechanical forces (bending, displacement,…) and parts of the membrane are transferred to the vascular system, there is a high risk of thrombosis or even embolism, maybe resulting in an infarct.

However, in the meantime two companies launched intravenous microdialysis probes. CMA Microdialysis AB launched their CMA64 IView catheter. Probe Scientific Ltd. launched their MicroEye®. Two clinical studies involving the CMA64 iView catheter have been published (O Rooyackers et al. 2010; Hage et al. 2010). They report that the accuracy was not sufficient and the variability between the recording periods was high without calibrations (O Rooyackers et al. 2010). In addition, the overall congruence between reference blood glucose concentration and intravascular microdialysis glucose was not acceptable in about 30% of the investigated subjects (Hage et al. 2010).

CMA Microdialysis' German distributor EKF-Diagnostic GmbH announced in March 2012 that the CMA64 iView catheter will no longer be available. They refer to their competitor Probe Scientific Ltd., whose product MicroEye® has not been used in a published clinical study so far – 5 years after product launch.

In addition to these peripheral venous approaches, two central venous iv-MD systems are currently developed and CE approved. The first one – DIRAMO – is developed by Flowsion AS. However, no publications are available on this system. The second central venous system is the EIRUS™ system, which was developed by CMA Microdialysis and is now marketed by Dipylon Medical. It features a specially designed multilumen central venous catheter with a semipermeable microdialysis membrane, which forms part of the outer catheter surface. The device also features a monitor and a biosensor, which requires calibration to plasma glucose concentration. Some studies were published recently (Christina Blixt et al. 2013; Schierenbeck et al. 2012; Schierenbeck et al. 2013; Möller et al. 2011) and the data suggest that the approach is promising in terms of accuracy and ISO criterion. EGA analysis revealed that 100% of the data pairs were in zones AB in all studies, and ISO criterion was met in more than 93% of all cases in all studies. However some of the published data were obtained using a prototype version of the EIRUS™ system with a single lumen central venous catheter (Schierenbeck et al. 2012; Christina

Blixt et al. 2013; Möller et al. 2011). Here the microdialysis probe is inserted into the lumen and protrudes from the catheter tip. One study has been published using the EIRUS™ triple-lumen catheter (Schierenbeck et al. 2013).

2.3 Extravascular microdialysis (ev-MD)

Another approach for vascular microdialysis is to perform blood microdialysis outside the body – extravascular microdialysis (ev-MD). Blood has to be transported from the patient to the dialysing membrane – like in hemodialysis – and can be re-infused thereafter. Dempsey et al. implemented this approach using a venous shunt (Dempsey et al. 1997, see **Figure 5**).

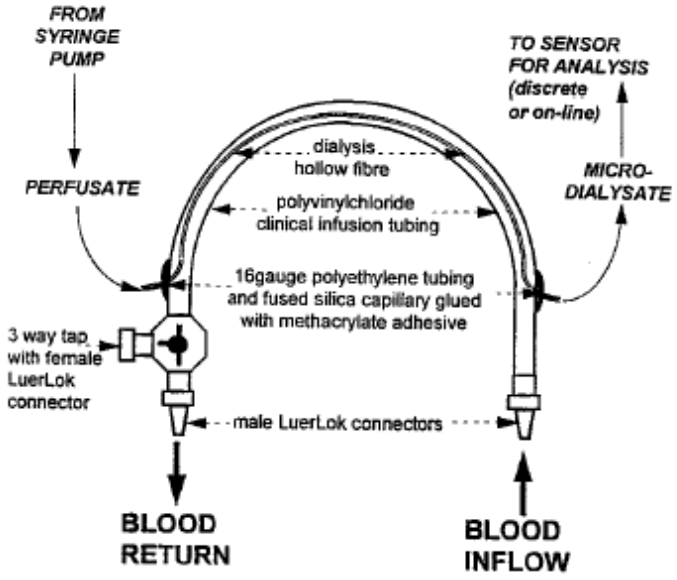


Figure 5: extravascular microdialysis probe as suggested by Dempsey et al.(Dempsey et al. 1997). Blood is pumped and reinfused through a venous shunt.

Transportation and re-infusion of blood is associated with some drawbacks of this method:

- blood has to be anti-coagulated and will therefore be diluted
- an additional pump is necessary to handle blood fluid transport, which makes this approach more complex compared to iv-MD
- the membrane and all other parts in contact with blood must be biocompatible to ensure safe re-infusion

On the other hand the advantages compared to iv-MD are:

- single catheter methods to continuously sample low volumes of blood are known from the prior art since the 1960s (Weller et al. 1960) and are implemented in state of the art glucose clamp devices (e.g. Biostator, Glucostator and Nikkiso STG-22)
- free choice of exchange area surface to optimise and ensure high and long-term stable recovery rates
- accessing the patient's vascular system is possible via existing ports of multi-lumen central/peripheral catheters
- no membrane fouling due to anti-coagulation of blood

3. Evaluating iv-MD and ev-MD

3.1 Risk assessment of the body interface approaches

A risk assessment was performed for the intravascular and the extravascular microdialysis approach by means of consulting professionals of different disciplines like hospital care and related sciences (biomedical engineers, medical doctors, biologists, chemists) on the two presented body interface approaches, aiming to identify potential technical failures and resulting harms for the patient.

Each identified failure was rated for severity of harm (rating 1-10: insignificant - critical) as well as for the probability of occurrence (rating 1-10: unimaginable - probable), and entered into a so-called 'ALARP table', which assigns each failure to an 'acceptable' (green), an 'ALARP – as low as reasonable possible' (green) or an 'intolerable' (red) region.

The results are shown in **Figure 6**. It is clearly shown that there is less risk associated with the extra-vascular approach compared to the intra-vascular even after having performed a re-evaluation, considering primary technical measures (e.g. material, sensors, alarms) and secondary measures (e.g. definition of application, user guidelines) to reduce certain risks.

Risk level (RS)			Severity of Harm (S)									
			Insignificant		Slight		Moderate		Severe			Critical
			1	2	3	4	5	6	7	8	9	10
Probability of Occurence (A)	Probable	10	0	0	0	0	0	0	0	0	0	0
	Occasional	9	0	0	0	0	0	0	0	0	0	0
		8	0	1	0	0	0	0	0	0	0	0
		7	0	0	0	0	0	0	0	0	0	0
	Marginal	6	0	1	0	0	0	0	0	0	0	0
		5	0	0	1	0	0	0	0	0	0	0
		4	0	0	0	0	0	0	0	0	0	0
	Unlikely	3	0	0	0	0	0	0	0	0	0	0
		2	0	3	0	0	1	0	0	0	1	0
	Unimaginable	1	0	2	0	0	0	0	0	1	0	1

Risk level (RS)			Severity of Harm (S)									
			Insignificant		Slight		Moderate		Severe			Critical
			1	2	3	4	5	6	7	8	9	10
Probability of Occurence (A)	Probable	10	0	0	0	0	0	0	0	0	0	0
	Occasional	9	0	0	0	0	0	0	0	0	0	0
		8	0	1	1	0	0	0	0	0	0	0
		7	0	0	1	0	0	0	0	0	0	0
	Marginal	6	0	0	0	0	0	0	0	0	0	0
		5	0	0	0	0	0	0	0	0	0	0
		4	0	0	0	0	0	0	1	0	0	0
	Unlikely	3	0	0	0	0	0	0	0	0	0	1
		2	0	0	0	0	0	0	0	0	1	0
	Unimaginable	1	1	1	0	0	0	0	0	0	0	2

Figure 6: Results of the risk analysis performed for the ev-MD (top) and the iv-MD approach (bottom).

3.2 Evaluation regarding economic aspects, technical feasibility and usability

In addition to the risk assessment several other categorised parameters have been evaluated aiming to obtain a decision basis which approach should be followed. Each individual criterion could be evaluated using a three grade system (0-2 points). All points of an evaluation category are summed up and weighted (technical evaluation: 15%, risk assessment: 30%, acceptance by doctors and nurses: 25%, economic evaluation: 25%, integration in CLINICIP system: 5%). The approach with the best grades (i.e. the least points) was chosen to be further followed.

3.3 Summary

The evaluation results are presented in **Table 1**. It is clearly shown that the extravascular microdialysis approach is favourable compared to the intravascular microdialysis approach (1.15 vs. 2.35 points). At the time the evaluation was performed iv-MD probes were not commercially available. Considering this aspect in a re-evaluation, would now also give 1.15 points for the iv-

MD approach (compare **Table 2**). However, taking into account that the CMA64 iView has been withdrawn from the market and taking into account, that only two publications are available using the iv-MD probe CMA64 iView, and no publications are available describing the use of the MicroEye probe, it is concluded that the ev-MD approach still is favourable compared to the iv-MD approach.

Table 1: Results of evaluating the extra- versus intravascular microdialysis approach

Evaluation Criteria for the vascular BI	Extravascular microdialysis	Intravascular microdialysis
TECHNICAL EVALUATION: 15%	1	4
Microdialysis performance evaluation	1	3
Acceptable High Recovery (to gain strong signal at sensors)	50% (stable)	Not tested, 50-90% not stable (fouling)
High effective diffusion surface (exchange area)	yes	limited membrane length
High blood/perfusate flow ratio (to prevent depletion of glucose in blood)	yes	yes
Membrane: Biocompatible, no fouling, platelet/protein adsorption: Coating, skin outside	yes (skin outside membrane + heparin)	yes, skin outside membrane possible
Chance of reliable long term operation = Reliability of constant dialysate supply, recovery stable for up to 100h	yes, see in vitro results	not for 100hours, according to literature
System integration: Bodyinterface + sensor	0	1
Acceptable delay time: <15min incl. Sensor, < 10min excl. Sensor (bedside: 1.5m patient safety distance)	calculated: 9.5min (4.5min blood tubing (150cm) + 5 min perfusate)	sensor must be directly located at perfusate outflow (catheter integration possible) ==> short delay time but sensor cannot be bedside
bedside integration (patient safety distance)	integration of all parts at the bedside is possible and prefereable. short delaytime despite bedside integration possible. blood withdrawal and sensor must not necessarily be directly at the patient.	sensor has to be at the patient to assure short delay time and thus is hindering the patient routine care, sensor miniaturisation is absolutely necessary
Sufficient dialysate delivery flow to reduce air bubble effects: flow > 1µl/min	yes	yes
Reinfusion of withdrawn blood	No, but blood loss is acceptable	No blood loss
Heparinzed blood	Yes, but no patient heparinization	no
Coated tubing system required	no	no
RISK ASSESSMENT: 30%	0	2
Result of risk analysis (Safe for patient: no acute dangers, risks acceptable)	3 of 13 evaluated risks in ALARP region for the improved setup	7 of 11 evaluated risks in ALARP region for the improved setup, according to risk analysis
ACCEPTANCE BY DOCTORS, NURSES (ergonomics, work load reduction, costs): 25%	1	1
Maintenance effort of BI+sensor set-up for operation acceptance under optimal circumstances (Acceptance ranking)	2: catheter blocking due to coagulation can occur more often compared to discontinuous blood withdrawal.	2: sensor located directly at patient, monitor is bedside, wireless communication not possible device is hindering when it is in the patient bed at the ICU Additional catheter is necessary and has to be set. No existing port or catheter can be used.
Inititalization time effort for staff compared to time effort with manual blood sampling 30 times/24hours	20min	20min
Short run in period - little calibration effort (Acceptance): ranking (1-3)	1	1
EVALUATION ACCORDING TO AN ECONOMIC PRODUCTION AND USE OF THE DEVICE: 25%	3	3
Complexity of the system setup: Ranking	2: complex fluidic transport necessary (perfustate, blood, heparin), but continuous	1: perfusate is the only fluid to be transported
regulatory affairs (regarding CE-labelling)	Bodyinterface is already CE-labelled	intravascular Microdialysis catheters have not been available at this time. Membrane must meet biocompatibility criteria. A complex and fagile device is inserted into the vascular system. In the meantime iv-MD probes are commercially available.
costs of disposable components (repeated costs, complexity,...): ranking (1-3)	2: estimated catheter costs of 50 EUR, pumps can be reused, estimated costs for planar flow through microdialysis device of 50EUR, heparin is additionally necessary	2: high catheter costs are expected (ca.150EUR: Assumption is based on other CE-labelled microdialysis catheters) but there is no need for further MD-equipment
Additional effort for safety monitoring: ranking (1-3)	2: heparin-blood-mixture-monitoring	1
MAJOR CRITERIA FOR INTEGRATION IN CLINICIP SYSTEM: 5%	0	3
Positive Risk Analysis (No risks in intolerable zone, minimum number in ALARP	yes	yes
Approval of ethics committee and authorities likely	yes, CE-labelled product in direct use with human	unlikely, not CE-labelled
Chance to prototype an integrated device within CLINICIP	probable	no: sensor integration directly at the patient impossible
RESULTS	**1,15**	**2,35**

Table 2: Results of re-evaluating the extra- versus intravascular microdialysis approach after commercialisation of CMA's iView and Probe Scientific's MicroEye

Evaluation Criteria for the vascular BI	Extravascular microdialysis	Intravascular microdialysis
TECHNICAL EVALUATION: 15%	**1**	**4**
Microdialysis performance evaluation	**1**	**3**
Acceptable High Recovery (to gain strong signal at sensors)	50% (stable)	Not tested, 50-90% not stable (fouling)
High effective diffusion surface (exchange area)	yes	limited membrane length
High blood/perfusate flow ratio (to prevent depletion of glucose in blood)	yes	yes
Membrane: Biocompatible, no fouling, platelet/protein adsorption: Coating, skin outside	yes (skin outside membrane + heparin)	yes, skin outside membrane possible
Chance of reliable long term operation = Reliability of constant dialysate supply, recovery stable for up to 100h	yes, see in vitro results	not for 100hours, according to literature
System integration: Bodyinterface + sensor	**0**	**1**
Acceptable delay time: <15min incl. Sensor, < 10min excl. Sensor (bedside: 1.5m patient safety distance)	calculated: 9.5min (4.5min blood tubing (150cm) + 5 min perfusate)	sensor must be directly located at perfusate outflow (catheter integration possible) ==> short delay time but sensor cannot be bedside
bedside integration (patient safety distance)	integration of all parts at the bedside is possible and prefereable. short delaytime despite bedside integration possible. blood withdrawal and sensor must not necessarily be directly at the patient	sensor has to be at the patient to assure short delay time and thus is hindering the patient routine care, sensor miniaturisation is absolutely necessary
Sufficient dialysate delivery flow to reduce air bubble effects: flow > 1µl/min	yes	yes
Reinfusion of withdrawn blood	No, but blood loss is acceptable	No blood loss
Heparinzed blood	Yes, but no patient heparinization	no
Coated tubing system required	no	no
RISK ASSESSMENT: 30%	**0**	**0**
Result of risk analysis (Safe for patient: no acute dangers, risks acceptable)	3 of 13 evaluated risks in ALARP region for the improved setup	not performed, but CE-labelled
ACCEPTANCE BY DOCTORS, NURSES (ergonomics, work load reduction, costs): 25%	**1**	**1**
Maintenance effort of BI+sensor set-up for operation acceptance under optimal circumstances (Acceptance ranking)	2: catheter blocking due to coagulation can occur more often compared to discontinuous blood withdrawal.	2: sensor located directly at patient, monitor is bedside, wireless communication not possible device is hindering when it is in the patient bed at the ICU Additional catheter is necessary and has to be set. No existing port or catheter can be used.
Inititalization time effort for staff compared to time effort with manual blood sampling 30 times/24hours	20min	20min
Short run in period - little calibration effort (Acceptance): ranking (1-3)	1	1
EVALUATION ACCORDING TO AN ECONOMIC PRODUCTION AND USE OF THE DEVICE: 25%	**3**	**1**
Complexity of the system setup: Ranking	2: complex fluidic transport necessary (perfustate, blood, heparin), but continuous	1: perfusate is the only fluid to be transported
regulatory affairs (regarding CE-labelling)	Bodyinterface is already CE-labelled	intravascular Microdialysis catheters have not been available at this time. Membrane must meet biocompatibility criteria. A complex and fagile device is inserted into the vascular system. In the meantime iv-MD probes are commercially available.
costs of disposable components (repeated costs, complexity,...): ranking (1-3)	2: estimated catheter costs of 50 EUR, pumps can be reused, estimated costs for planar flow through microdialysis device of 50EUR, heparin is additionally necessary	2: high catheter costs are expected (ca.150EUR: Assumption is based on other CE-labelled microdialysis catheters) but there is no need for further MD-equipment
Additional effort for safety monitoring: ranking (1-3)	2: heparin-blood-mixture-monitoring	1
MAJOR CRITERIA FOR INTEGRATION IN CLINICIP SYSTEM: 5%	**0**	**1**
Positive Risk Analysis (No risks in intolerable zone, minimum number in ALARP zone)	yes	yes
Approval of ethics committee and authorities likely	yes, CE-labelled product in direct use with human	unlikely, not CE-labelled In the meantime iv-MD probes are commercially available.
Chance to prototype an integrated device within CLINICIP	probable	no: sensor integration directly at the patient impossible
RESULTS	**1,15**	**1,15**

4. Extravascular microdialysis approaches

To perform extravascular microdialysis blood has to be transported from the patient to the microdialysis device. Thus an ev-MD system consists of two subsystems: one for continuous blood withdrawal and the microdialysis device itself.

4.1 Continuous blood withdrawal using a double lumen catheter

A double lumen catheter (DLC; mtb GmbH, Lonsee, Germany) is inserted into a peripheral vein. The outer lumen of the catheter is continuously flushed with a NaCl-Heparin solution at a flow rate of 2ml/h to prevent coagulation (heparin concentration: 50 IU/ml). At the tip of the DLC heparinised blood is withdrawn through the second lumen at a flow rate of 4ml/h. Thus net blood withdrawal is 2ml/h. This technique was first used by Weller et al as early as in 1960 in a clinical glucose monitoring system (Weller et al. 1960). Today it is used in the Biostator and the Glucostator glucose clamps devices. A picture of the DLC can be found in **Figure 7**

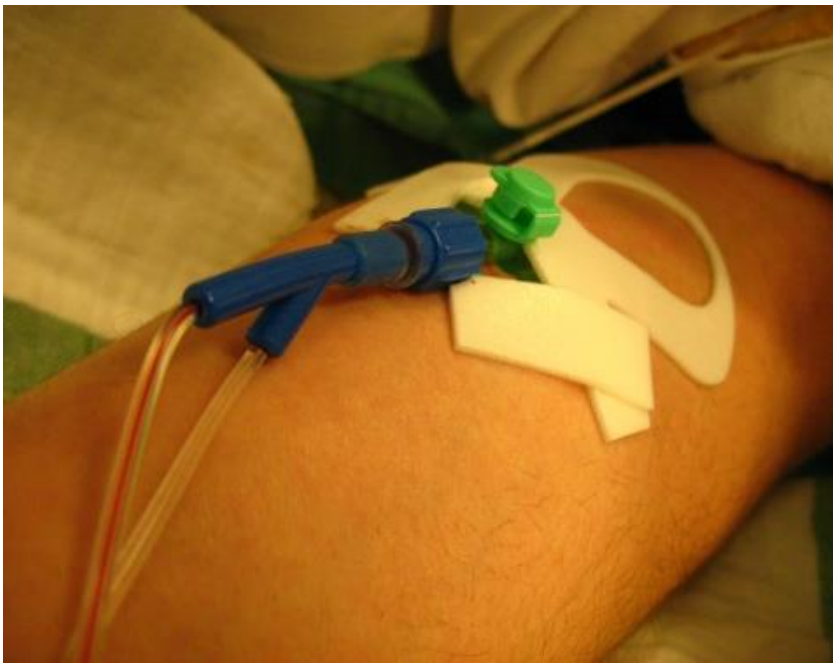


Figure 7: Double lumen catheter (DLC). Lumen 1: heparin infusion; Lumen 2: Blood+heparin withdrawal

The Weller-approach was adapted and some features were implemented to minimise coagulation problems and to increase safety:

- Backflux of potentially contaminated blood from the waste compartment is prevented by using peristaltic pumps uni-directionally.

- Blood and heparin were simultaneously withdrawn from the subjects via the outer lumen of the double lumen catheter. Therefore no heparin is infused into the subjects. For safety reasons the administration-flow of heparin was monitored by a pressure transducer placed between heparin pump and double lumen catheter (see **Figure 8**, blue line). Unintended heparin infusions would have triggered an alarm. To control the subject's coagulation level in the clinical trials, additional blood samples were taken every 5 hours to determine their APTT level. During the whole study period no unintended heparin infusion was detected.
- To prolong catheter patency intermittent flushing sequences were applied. Each 30min the flow rate of the NaCl-heparin solution was increased to the five-fold for 10 seconds, resulting in a short NaCl-heparin bolus infusion into the subject's vessel (see **Figure 8**, blue peak). The total amount of thereby infused heparin units is 0,69IU per bolus infusion giving a maximum total amount of 41,67IU being administered into the subject's vein during a 30-hour trial.
- An optical sensor was integrated in the blood tubing before the entrance to the flow through microdialyser unit. This sensor signal was used to determine unintended blood dilutions as well as vein occlusions or blockings of the DLC. In **Figure 8** the green line represents the blood detector signal. It can be seen at the short green peak after the heparin flushing sequence (blue peak) that blood is being diluted by this heparin flushing.

Pumps, sensors and valves were PC- controlled using Lab VIEW® 7.0 software on a notebook and a NI 9263 4-Channel 16-Bit Analog Voltage Output Module (all from National Instruments, Inc., Austin, TX, USA) (Franz Feichtner et al. 2010). The developed Lab VIEW program was also used to visualise the system's status (alerts, signals) on a graphical user interface (see **Figure 9**).

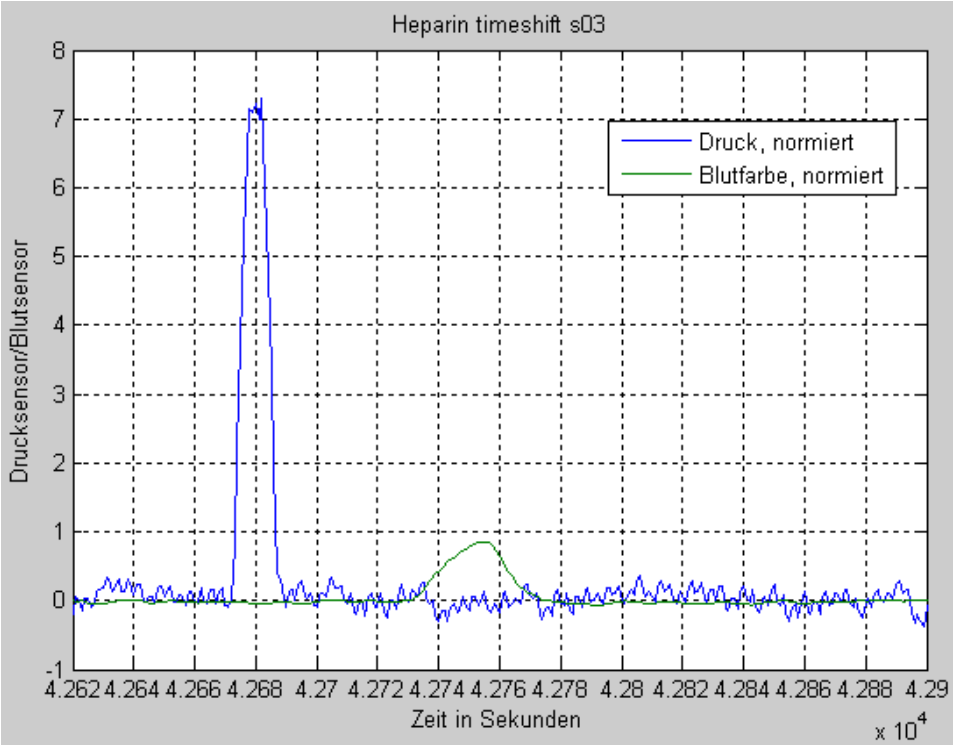


Figure 8: Exemplary pressure (blue) and optical sensor (green) profiles of the continuous blood sampling system. The pressure signal was recorded in the heparin infusion line to monitor unintended heparin infusion. The blue peak represents an intended, 10 seconds lasting, increased heparin infusion, which was applied each 30 minutes to prolong catheter patency. The green line is the optical blood detector signal, which was recorded inside the blood withdrawal line to determine unintended blood dilutions as well as vein occlusions or blockings of the DLC. It can be seen at the short green peak after the heparin flushing sequence (blue peak) that blood is diluted by this heparin flushing.

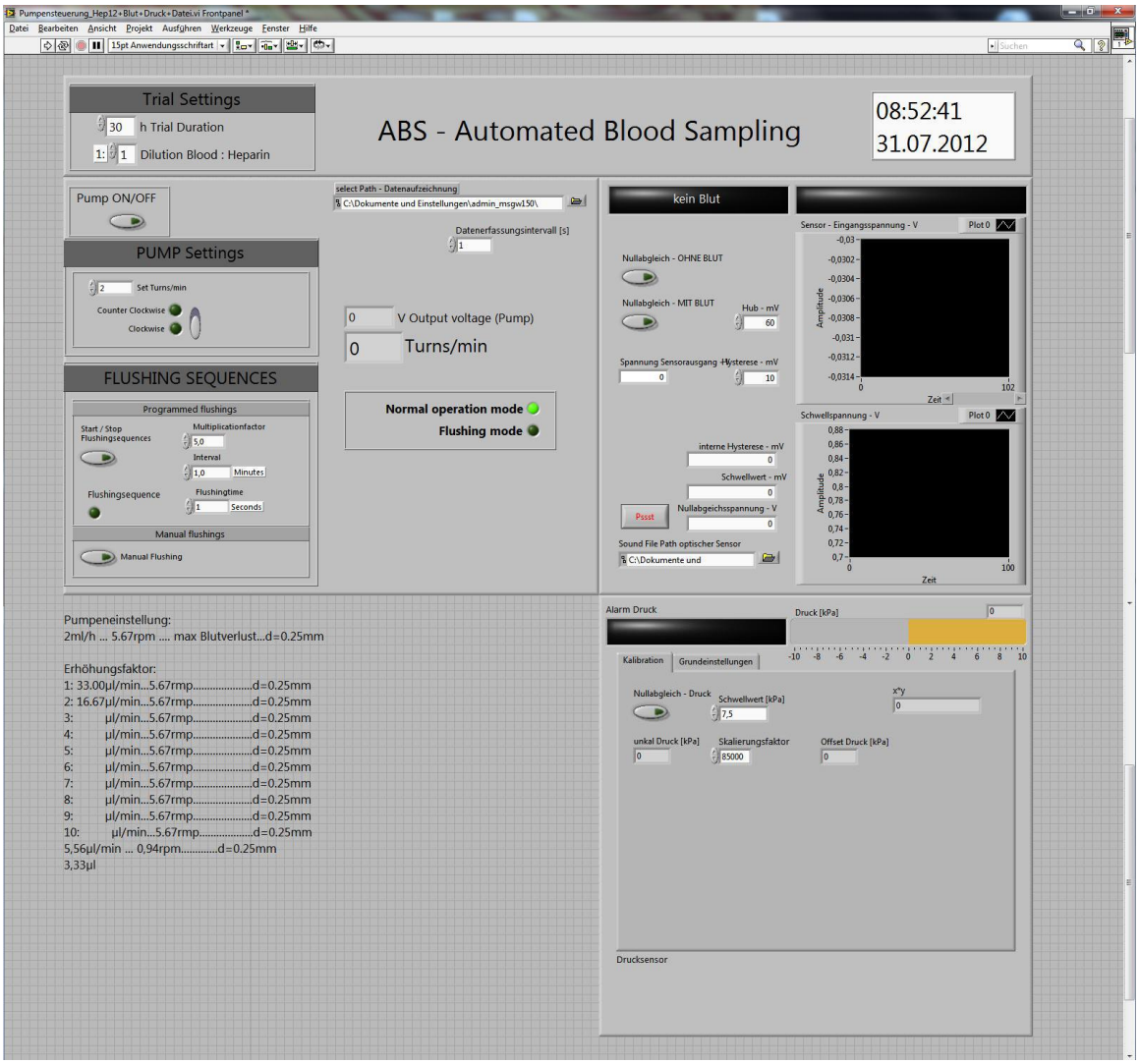


Figure 9: Graphical user interface of the developed LabVIEW-based software to control pumps, sensors and valves and to visualise the system's status (alerts, signals) of the continuous blood sampling system.

4.2 Extravascular MD approach 1: Cartridge approach

Following Dempsey at al's approach (Dempsey et al. 1997) a first attempt to do extravascular micordialysis was designed. A conventional hemodialyser was mimiced, yet miniaturised. A bundle of conventional hollow dialysis membranes was inserted into a mini-cartridge, which itself was counter-currently perfused with perfusate and blood as depicted in **Figure 10**.

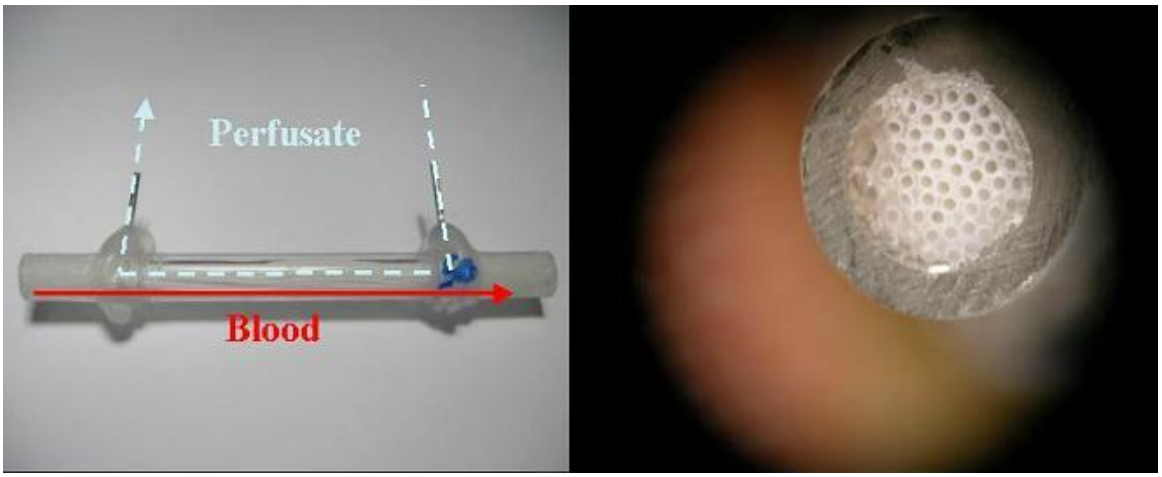


Figure 10: First extravascular MD approach using a mini-cartridge with a bundle of hollow dialysis fibres. Left: Cartridge with schematically illustrated counter current operation mode. Right: Cross section of the cartridge with a bundle of hollow fibres.

4.2.1 Material and methods

Recovery, long term performance and response time have been investigated using different glucose concentrations (2.7, 5.5 and 10 mM glucose) in glucose enriched ELOMEL solution and anti-coagulated and glycolysis-inhibited bovine blood. Different types of mini-cartridges have been tested, employing different number of fibres (40 and 60 fibres; length: 5cm) and different methods of membrane packing inside the cartridge. Ion-free perfusate was pumped with different flow rates (10, 25, 50 and 100µl/min) whereas the test solution was always pumped through the membranes at 250µl/min.

4.2.2 Results

The results of these in-vitro tests were not promising, however:

- The maximum glucose recovery was 82%, achieved at the lowest perfusate flow rate (10 µl/min), a glucose solution flow rate of 250 µl/min and using 60 fibers in a bundle (see **Figure 11**. The recovery for the cartridge approach was not as high as initially expected. Though the theoretical exchange surface was extraordinary high using this multi-fibre approach, the effective exchange surface might be much lower. It can be speculated that the perfusate takes the shortest way from inlet to outlet (compare **Figure 10**), without being exerted to all fibers in the bundle.
- In **Figure 12** absolute dialysate glucose concentrations (red) were calibrated using the ionic-reference technique (Lukas Schaupp et al. 1999). Sodium concentration in the test

solution was known and stable and sodium concentration in the dialysate was measured at each flow. The ratio between the dialysate sodium concentration and the sodium concentration in the test solution was taken as “online calibration factor”. At low perfusate flow-rates (high recovery, calibration factor close to 1) the dialysate glucose and the calibrated glucose concentration are comparable. At increased flows Ionic reference technique could not compensate for the decreasing glucose recovery. It is speculated that sodium recovery fell to a stable level of 70% at higher perfusion flow rates.

- The cartridge had a considerable dead volume of approximately 200µl resulting in a pronounced response time of more than 20 minutes to detect concentration changes, which was unacceptably high. The ratio between the dead volumes of the blood and the perfusate compartments could not be optimised as to reduce response time while at the same time maximise recovery.
- It was impossible to reproducibly manufacture these cartridges manually
- Stagnant zones in the cartridge abated blood coagulation

4.2.3 Conclusion

It was concluded that the cartridge approach with a bundle of fibres within a concentric lumen was not an appropriate way to perform extravascular microdialysis. Geometry and flow conditions had to be optimised.

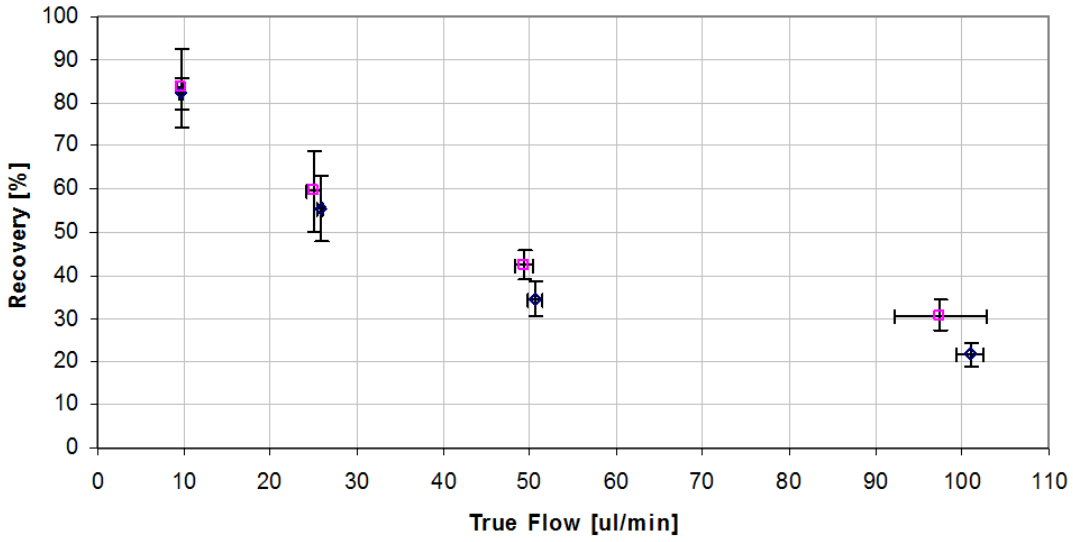


Figure 11: Glucose recovery of extravascular body interface based on a cartridge approach. Cartridges with 40 (red) and 60 (blue) fibres were perfused at different perfusion flow rates.

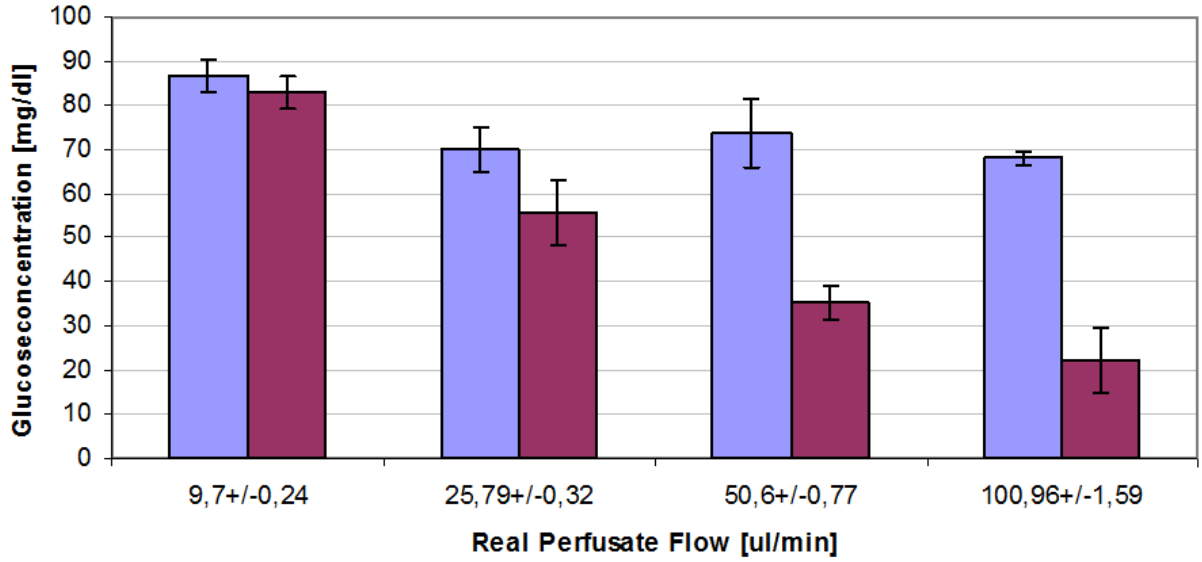


Figure 12: Mean glucose concentrations of the extravascular body interface based on a cartridge approach. Cartridges with 40 fibres; red: uncalibrated dialysate glucose concentration; blue: corrected dialysate glucose concentration using the ionic reference technique (Lukas Schaupp et al. 1999)

4.3 Extravascular MD approach 2: Planar flow-through MD (PFTMD)

In search for an easily reproducible ev-MD approach with reduced overall and dead volume, no stagnant zones, and highest possible recovery (high exchange surface) another approach using planar microdialysis membranes was followed. This geometry promised to achieve high recoveries and short response times.

In an in-vitro study the long-term recovery stability and response time was investigated to find optimal operating conditions. In vivo performance was investigated in a clinical pilot study.

4.3.1 Material and methods

Two sterilisable teflon plates with three 70µl microfluidic channels on its surface are separated by a planar membrane (provided by Gambro, MWCO ~10kDa) to form three independent planar flow through microdialysis cells. Blood and perfusate were pumped through these two cells (see **Figure 13**). The teflon plates and the holder were provided by Harvard Apparatus.

In-vitro evaluation

During a 72hour experiment the channels were perfused with temperature controlled (37°C) bovine blood at 2ml/h on the one side and with 5% Mannitol as perfusate (Fresenius Kabi, Graz) on the other side at 2, 3, 5 and 10µl/min in counter current direction. Bovine blood was anti-

coagulated using 500 mg potassium oxalate per 1,000 ml blood and glycolysis-inhibited by using 750 mg sodium fluoride per 1,000 ml blood. Blood glucose concentration was spiked to two concentration levels, 90 and 130mg/dl, which were alternately used as test solution each 4 hours. At times when the test solution was changed the response time of the system was measured as rise- and falltime in the online glucose signal, which was obtained using an optical glucose sensor (Pasic et al. 2006).

In-vivo evaluation

A preliminary ten-hour in vivo investigation was performed in one subject. Blood was withdrawn using two double lumen catheters). After a 3 hour run in period an oral glucose tolerance test (OGTT) was performed using 75g of glucose. Unintended subject heparinisation was monitored by APTT determination before, during and after the experiment. Glucose concentration in dialysate outflow and in centrifuged plasma samples from the reference catheter was determined by Beckman Glucose Analyser.

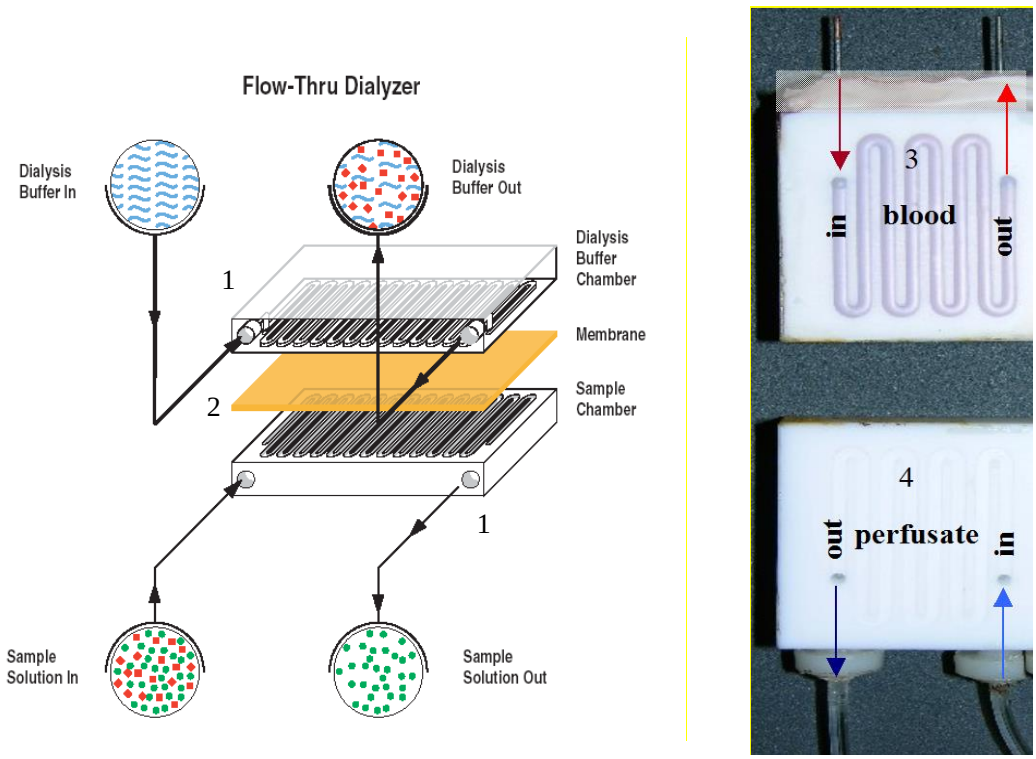


Figure 13: Planar flow through microdialysis device (Harvard Apparatus): Schematic illustration (left); Teflon plates with microchannels and indicated flow directions (right)

4.3.2 Results

- Glucose recovery was found to range between 80% and 105% in the 72hour in-vitro experiment with a maximum at a perfusate flow rate of 2µl/min and a minimum at 10µl/min (see **Figure 14**).
- The response time was found to be around 13 minutes, which is much faster compared to the cartridge approach, but which is still too high (see **Figure 15**).
- **Figure 16** shows the one point calibrated dialysate signals (bright and dark violet line) and the reference blood glucose concentration (red line). Dialysate signals are discontinuous, because the double lumen catheter clogged and had to be flushed several times. Before and after this intervention the catheter worked well.

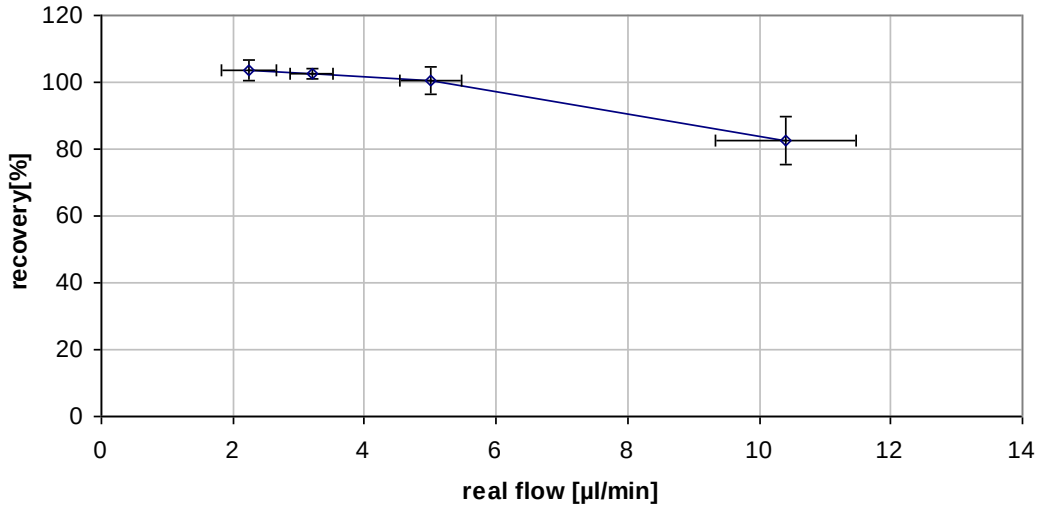


Figure 14: Glucose recovery data of a planar flow through microdialysis device with three independent planar flow through microdialysis cells at different perfusate flows conditions tested in a 72h experiment with bovine blood as test substance.

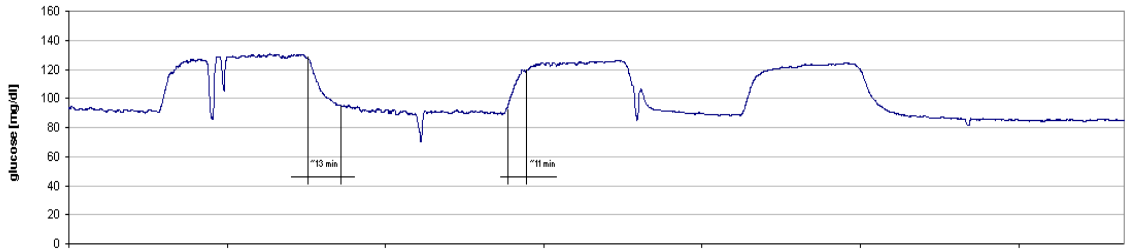


Figure 15: online glucose signal measured with an optical glucose sensor (Pasic et al. 2006) to determine system response time

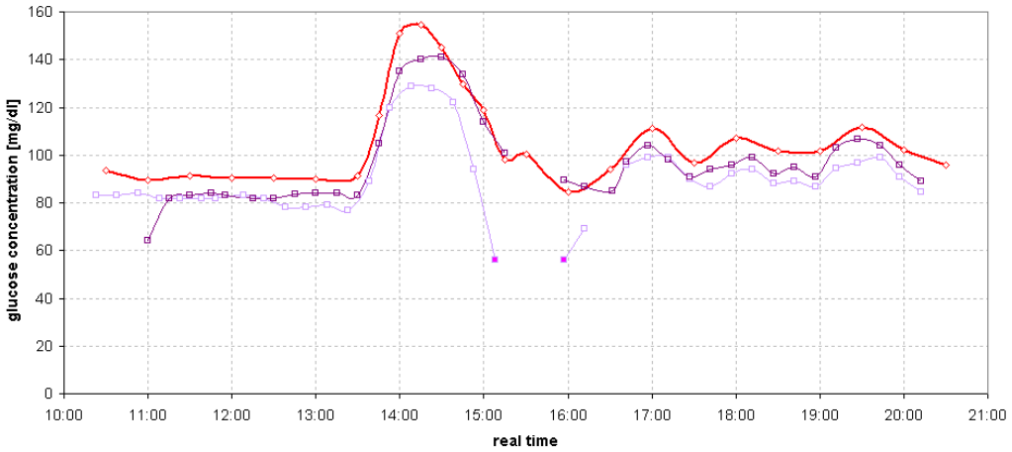


Figure 16: Reference (red) and two dialysate (dark and light violet) glucose concentration profiles

4.3.3 Conclusions

- High and long term stable recovery can be achieved for perfusate flow rates of < 5µl/min
- System response time is 13 minutes. Dispersion of blood in the tubing and thus response time might be reduced if tubing length and diameter were further reduced
- PFTMD cells are easy to use, mechanically robust and easy to sterilise via EtO
- Coagulation problems occurred in the DLC and new strategies for blood withdrawal had to be investigated
- The volume of the microfluidic channels in the dialysis cell is quite high, which is estimated to contribute with approx. 30% to the current delay time
- A re-design of the flow through cell was anticipated to further reduce delay time

4.4 Extravascular MD approach 3: Re-design of the planar flow-through MD

The geometrical design of the PFTMD was further optimised with regard to minimise dead volumes and with regard to avoide stagnant zones (e.g. edges or orthogonal changes in flow direction) to minimise the probability of blood coagulation.

The re-designed PFTMD (Franz Feichtner et al. 2010) consists of two polycarbonate plates that meet biocompatibility ISO 10993-1 standard (37×33×8 mm, Makrolon Rx-1805 from Bayer AG, Leverkusen, Germany, see Figure 17). They sandwich a semi-permeable membrane in between. On the plate's surfaces meander-like microfluidic channels are engraved with a volume of 8 µl and an active diffusive surface of about 90 mm². The PFTMD is a multi-use device. Its

polycarbonate plates are sterilisable using steam. Perfusate or blood is connected to the plate via HPLC screw connectors.

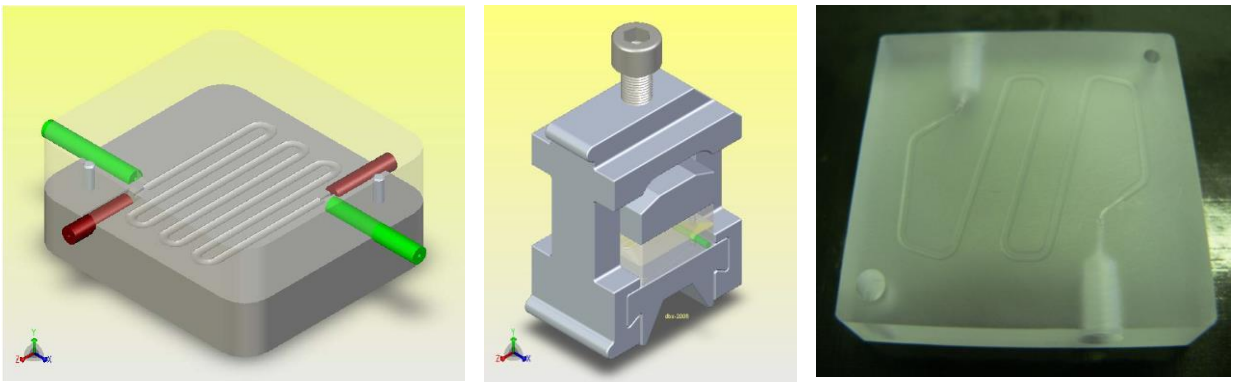


Figure 17: left: Schematic drawing of a redesigned microfluidic plate with engraved microfluidic channels; middle: holder system; right: picture of the redesigned microfluidic plate

In pre-clinical and clinical studies the first plate is connected to the DLC with TYGON® tubing (S-50-HL, ID=0.25 mm, OD=0.75 mm, l=1,500 mm, Saint-Gobain Performance Plastics, Beaverton, France) using re-usable custom made HPLC screw connectors and perfused with heparinised blood. The other plate is connected to a 5% Mannitol solution (Fresenius Kabi, Graz, Austria) with TYGON® tubing (R-3606, ID=0.19 mm, OD=2.01 mm) and is perfused counter-currently. Plates and connectors were steam sterilised at 121°C for 20 min before each experiment. Tubing and membrane are single-use only and were EtO sterilised before use. A schematic illustration of the complete system including the continuous blood sampling system is shown in **Figure 18**.

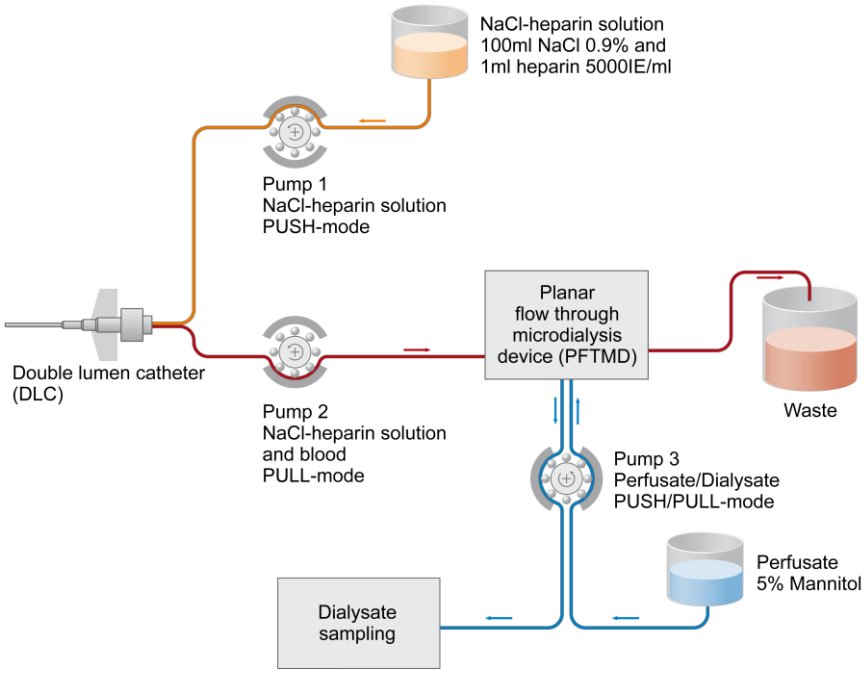


Figure 18: Schematic illustration of the extravascular microdialysis approach 3, which is based on a planar flow through microdialysis device and a system for continuous blood withdrawal (Franz Feichtner et al. 2010).

4.4.1 Pre-clinical evaluation[2]

Five PFTMDs were tested in vitro in 72 h experiments to find optimum operating conditions with respect to achieve high relative recovery while having short transport delay times. Tests were performed using anticoagulated, glycolysis-inhibited and temperature controlled (37°C) bovine blood as test matrix (anticoagulation: 500 mg potassium oxalate per 1,000 ml blood, glycolysis inhibition: 750 mg sodium fluoride per 1,000 ml blood) and 5% Mannitol as perfusate. Bovine blood flow rate was fixed at 4 ml/hour, whereas perfusate flow rate was varied between 2, 3, 5 and 10 µl/min. Reference blood samples (100 µl) were taken directly from the blood pool in hourly intervals. Continuously withdrawn blood samples were collected at the outflow of the PFTMD in 100 µl fractions. Both were centrifuged and supernatant plasma was collected for glucose analysis. Dialysate samples were collected at the dialysate outflow of the PFTMD in 25 µl fractions. All samples were frozen at −80°C for subsequent glucose analysis with a Roche Cobas Mira analyser using Cobas Gluco-quant and Glucose/Hexokinase (Roche Diagnostics GmbH, Mannheim, Germany).

[2] Partly taken and adapted from (Franz Feichtner et al. 2010)

In vitro investigation in bovine blood revealed that the mean relative glucose recovery level remained stable at a level of around 100% for 72 h at perfusate flows between 2–5 µl/min and a blood flow of 4 ml/min. Detailed results are given in Table 1. Relative recovery of 100.4±4.2% was achieved at a perfusate flow of 5 µl/min. This flow is regarded as the optimal perfusion flow rate combining complete relative recovery at a perfusion rate yielding in acceptable low system transport delay time. Thus, in the following clinical investigation 5 µl/min was chosen as perfusion flow rate.

Table 3: Relative recovery levels at different perfusate flow rates in 72 h in vitro MD experiments using five PFTMD devices, custom made MD membranes and heparinised bovine blood at a flow of 4 ml/h.

Perfusate flow [µl/min]	Relative recovery [%]
2	103.6±4.3
3	102.6±2.1
5	100.4±4.2
10	82.7±6.9

4.5 Membrane development[3]

To overcome the limitations of the reported decreasing blood microdialysis performance over time (see chapter on “Principles of iv-MD”) it is vital to develop a membrane having the selective layer with a smooth surface on the blood contacting side. High-porous hydrophilic PAES membranes were prepared by Gambro (Hechingen, Germany) by phase separation. The membrane features a weight cut-off of 10 kDa, thickness of 60 µm and a liquid permeability of 2-3×10^{-4}cm³/(cm² sec bar)). It combines high diffusive and controlled convective transport characteristics. Its extremely “smooth” and “low” thrombogenic selective layer is on the blood side with selective pore diameters between 2–10 nm, reducing the probability of cell and protein adhesion to the membrane surface and thus prevents a decrease of dialysis efficiency. So

[3] Partly taken and adapted from (Franz Feichtner et al. 2010)

high molecular weight proteins won't interfere / disturb the sensing device. A scanning electron micrograph showing a cross section of the microdialysis membrane is shown in Figure 19.

The membrane's production method is intellectually protected by a granted patent of Gambro (Krause et al. 2006) as is the membrane itself and its application in (vascular) microdialysis by a granted patent of the author and co-workers (Franz Feichtner et al. 2006).

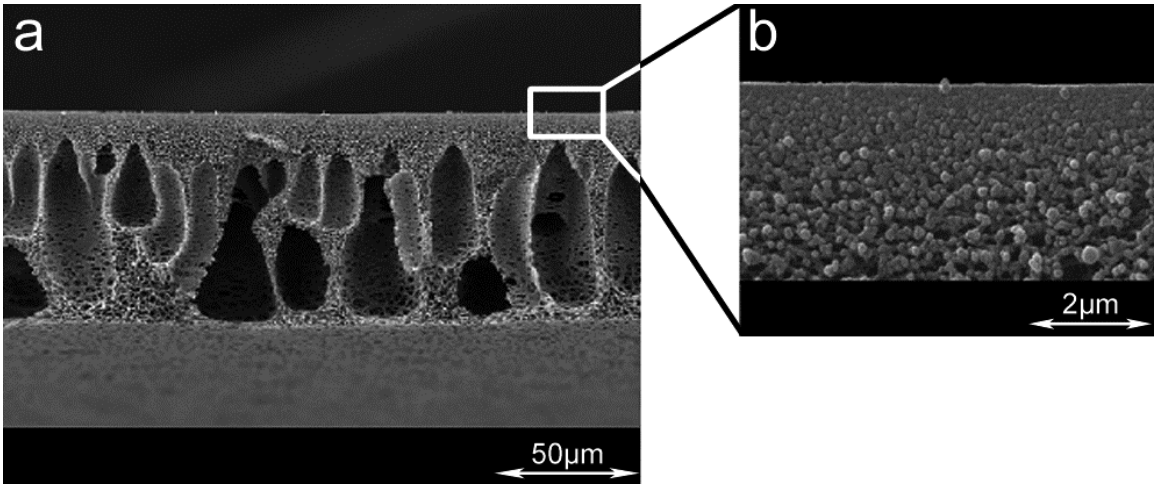


Figure 19: Scanning Electron Micrographs of a hydrophilic microdialysis membrane's cross section: (a) Whole cross section of the flat sheet membrane (Magnification: 600). (b) Active separation layer (Magnification: 20.000). The selective layer on top shows a narrow pore size distribution (Franz Feichtner et al. 2010).

5. Summary and conclusions

The herein presented approach of a vascular microdialysis system based on a combination of continuous blood withdrawal and an extravascular planar flow-through microdialyser was regarded the most promising body interface approach for a continuous glucose monitoring system based on blood measurement. Various advantages of current state-of-the-art techniques were combined. Direct access to blood enables to continuously monitor the systemic glucose concentration and performing microdialysis outside the patient is safer than placing a fragile membrane into the vascular system.

To overcome the described drawback of decreasing efficiencies of microdialysis membranes that are exposed to blood, also a new membrane with a very "smooth" blood contacting surface was developed, onto which blood clot formations are very unlikely to adhere. In contrast to reported recovery degradation following probe implantation into a blood vessel (Chen & Steger 1993; Yang et al. 1997; Verbeeck 2000; Yokel et al. 1992) it was found that the glucose recovery levels remained stable in these investigations using a newly designed membrane. It may therefore be

concluded that the membrane is superior to those presented in the prior art with respect to recovery stability when exposed to heparinised blood (Franz Feichtner et al. 2010).

Results of the in vitro tests demonstrated that high relative recoveries were possible despite having a quite fast perfusion flow rate of 5µl/min. This was achieved by minimising dead volumes in the microdialyser and thus system transport delay times.

After these technical in vitro performance and characterisation tests, in vivo tests in healthy and type-1 diabetic volunteers were performed. They are described in detail in the next chapters.

The concept of performing extravascular microdialysis in combination with a membrane having a very smooth blood contacting surface was patented by the author and co-workers (Franz Feichtner et al. 2006) and was published together with in vitro results of this chapter and with first in-vivo results of the following chapter.

CHAPTER III

Technical and clinical evaluation of the extravascular microdialysis based body interface for continuous blood glucose monitoring in a clinical study

This chapter is partly taken from a previously published article (Franz Feichtner et al. 2010) and is complemented by so far unpublished data

1. Introduction

In the previous chapter the design, development and implementation of a body interface for continuous blood glucose monitoring was presented. This chapter now describes the first clinical evaluation tests. The objectives were to

- find optimal operating conditions after the preclinical experiments
- perform a technical evaluation of the system
- perform a clinical performance evaluation and to record time concentration profiles of manually withdrawn blood (reference), dialysate and automatically withdrawn venous blood
- evaluate the correlation between the concentrations of glucose in manually withdrawn venous blood samples (reference) and dialysate
- identify potential for technical improvements

2. Material and methods[4]

The study was performed according to Good Clinical Practice (GCP) guidelines at the Clinical Research Centre located at the Medical University of Graz. Ethical approval was obtained from the local ethical committee. Signed informed consent was obtained from each subject before any trial related activities. Eight healthy non-diabetic volunteers (7 males, 1 female; age: 28.9±3.5 years; BMI: 25.1±1.5 kg/m²) were included.

2.1.1 Study protocol

Subjects arrived in the morning of the trial in a fasting condition. A peripheral 20 Gauge venous catheter (CODAN pvb Medical GmbH, Lensahn, Germany) and the DLC were applied at the Vena mediana cubiti of the right and the left arm of the subjects for reference and continuous blood sampling, respectively. The DLC was connected to the PFTMD and continuous blood sampling was performed as described above. After this setup procedure the study protocol started (sampling interval: 30 min). After an initial 5h fasting period, the subjects underwent an oral glucose tolerance test using 75 g glucose to test the system over a dynamic range of glucose

[4] Partly taken and adapted from (Franz Feichtner et al. 2010)

values. Thereafter 3 h of highly dynamic glucose levels were expected (OGTT period, sampling interval: 15 min), which was followed by a 4-h post-OGTT period (sampling interval: 30 min). The trial ended after 12 h.

2.1.2 Sampling

Reference blood samples (REF, 100 µl) were taken from the reference catheter, centrifuged and supernatant plasma was collected. Microdialysate samples (DIA) were collected for 15 min to get enough sample volume for glucose analysis and were vortexed afterwards. Thus, DIA samples reflect a time integrated glucose concentration. REF samples were taken in the middle of each DIA sampling period. All samples were immediately analysed for glucose concentration using a Beckman glucose analyser.

Additionally, three blood samples were taken from the reference catheter for determining the activated partial thromboplastin time (APTT) at 0 h, 5 h and 12 h in order to verify the absence of any significant patient heparinisation.

2.1.3 Data analysis

A **technical evaluation** was performed according to Wentholt et al. (Wentholt et al. 2008), including analysis of relative recovery, Bland and Altman analysis, Pearson's coefficient of correlation (R), absolute (AD) and relative differences (RD), mean absolute relative difference (MARD) and mean relative difference (MRD) and %PRESS (Lodwig & Heinemann 2003). ISO criterion is met if the system's glucose concentration is within ±15 mg/dl or within 20% of the reference glucose concentration, for glucose concentrations <75 mg/dl and ≥75 mg/dl, respectively. REF and DIA data were splined on minute basis and cross-correlated in a ±20 min time window to determine transport delay time which was assumed to be found most likely at the maximum occurring coefficient of correlation. DIA data were prospectively one point calibrated using the first valid DIA and corresponding REF sample.

Clinical evaluation included Clark Error Grid analysis (Clarke et al. 1987) and Insulin Titration Error Grid Analysis (Ellmerer et al. 2006). Clinical performance evaluation was performed using not delay-time corrected DIA data, as they would also not be time corrected in a clinical setting. The EGA is a standard method to classify data pairs of a new glucose sensing method and a reference method into zones with different levels of clinical acceptability; the ITEGA is an adaption of the EGA and was developed especially for intensive care patients undergoing intensive insulin therapy.

Data analysis was performed using Matlab (The Math-Works, Inc., Natick, MA, USA) and Microsoft Excel (Microsoft, Inc., Redmond, WA, USA).

3. Results[5]

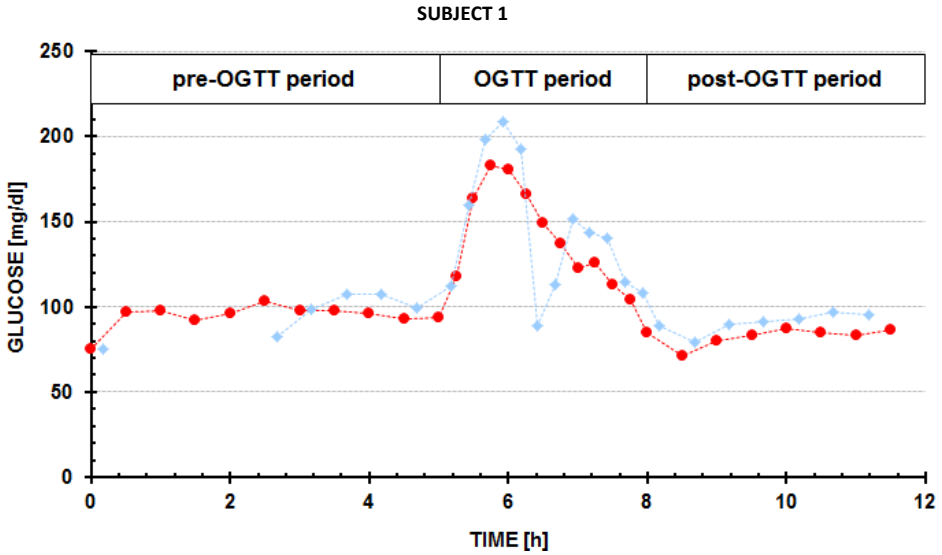
3.1 Technical performance evaluation

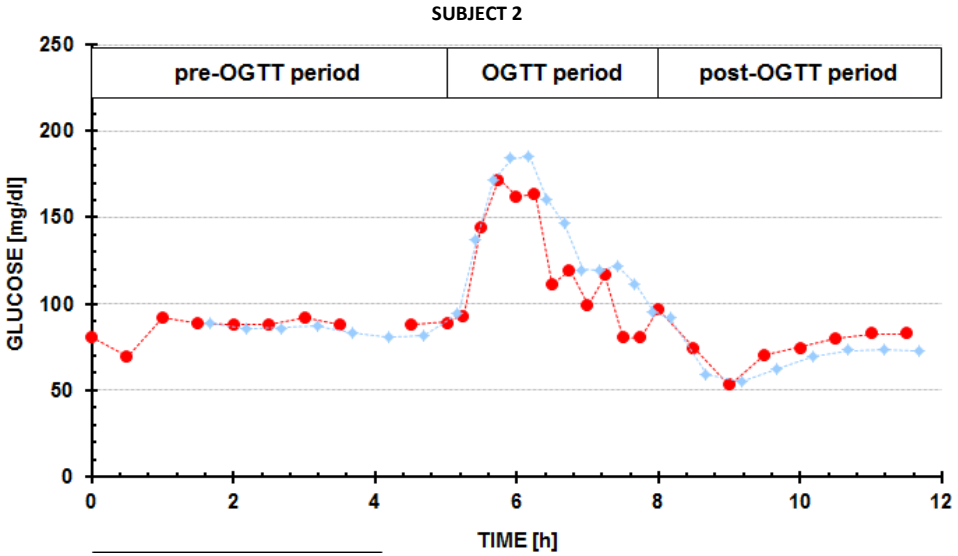
- Blood-heparin withdrawal was monitored in all eight subjects and was found to be 66.9±5.6 µl/min.
- The system's mean lag time due to blood + dialysate transport was found to be 10.5 min.
- Glucose concentrations ranged between 53.0 and 213.7 mg/dl (<80 mg/dl: 31 samples, 80–120 mg/dl: 139 samples, >120 mg/dl: 69 samples).
- 13 DIA samples (5.4%) were not taken due to malfunction of the system, mostly as a result of catheter occlusion.

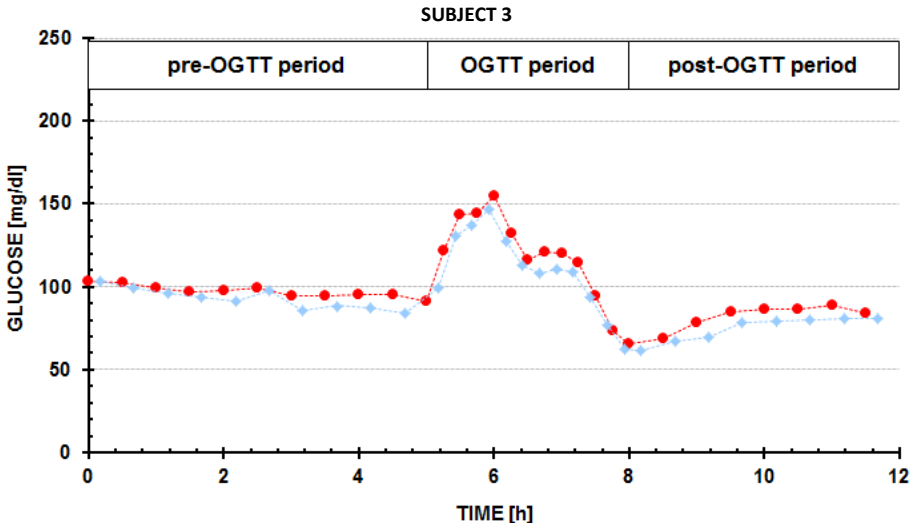
Figure 20 shows the glucose concentration profiles of all investigated subjects, derived from reference (REF, red) and microdialysis based system (DIA, blue). Microdialysis samples were prospectively one-point calibrated to the first reference blood glucose concentration and their glucose concentrations are corrected by the calculated lag time.

Technical data evaluation according to Wentholt et al. (Wentholt et al. 2008) is summarised in Table 4. Mean coefficient of correlation (CORR) was found to be 0.96±0.042. Mean difference (MD) was 2.1±12.1 mg/dl. Mean absolute difference (MAD) was 8.7±8.6 mg/dl. Mean relative difference (MRD) was 1.9±11.2%. Mean absolute relative difference (MARD) was 8.4±7.7%. %PRESS parameter was calculated as 10.3±5.2%. ISO criterion (ISO MET?) was met in 91.6% of all cases. Four out of eight trials were performed with 100% success rate (ISO criterion met). Bland and Altman analysis on transport-time-delay corrected DIA and REF data are provided in Figure 21.

[5] Partly taken and adapted from (Franz Feichtner et al. 2010) and complemented by so far unpublished data


SUBJECT 1
pre-OGTT period
OGTT period
post-OGTT period
GLUCOSE [mg/dl]
TIME [h]
0
50
100
150
200
250
0
2
4
6
8
10
12


SUBJECT 2
pre-OGTT period
OGTT period
post-OGTT period
GLUCOSE [mg/dl]
TIME [h]
0
50
100
150
200
250
0
2
4
6
8
10
12


SUBJECT 3
pre-OGTT period
OGTT period
post-OGTT period
GLUCOSE [mg/dl]
TIME [h]
0
50
100
150
200
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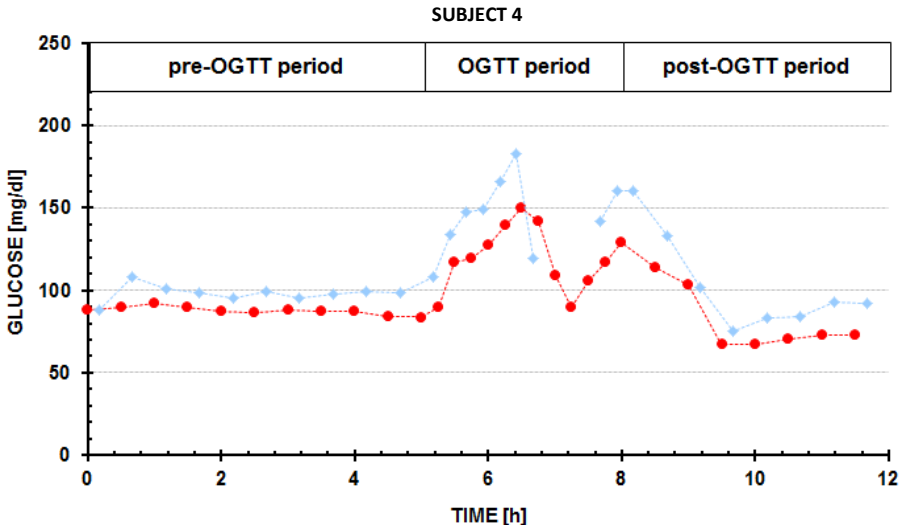
0
2
4
6
8
10
12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pre-OGTT period
OGTT period
post-OGTT period
GLUCOSE [mg/dl]
TIME [h]
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100
150
200
250
0
2
4
6
8
10
12

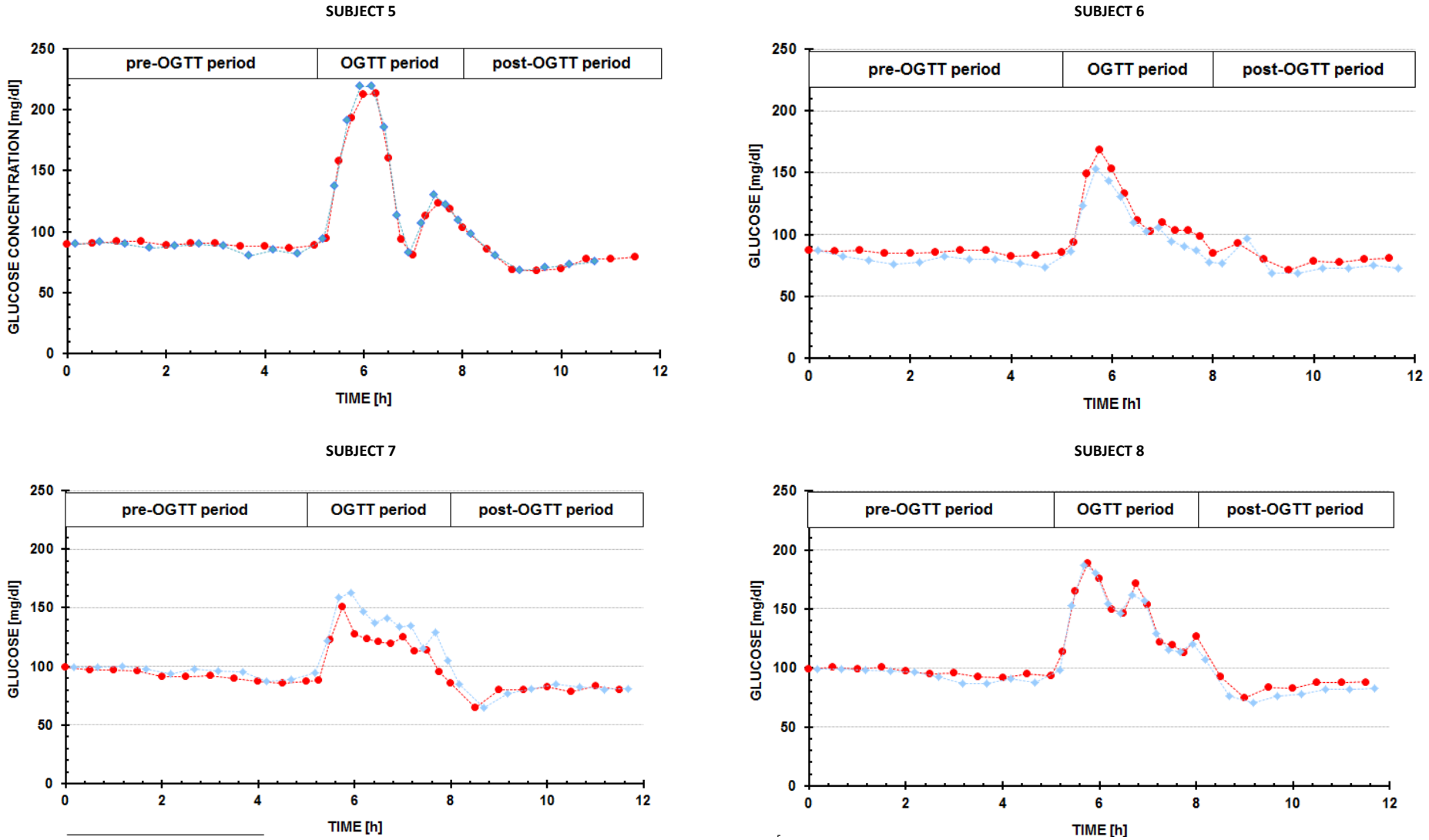

Figure 20: Glucose time-profile showing reference (REF, red) and prospectively one point calibrated, transport-time-delay-corrected glucose concentration of the microdialysis based device (DIA, blue) for continuous extravascular monitoring of blood glucose concentration

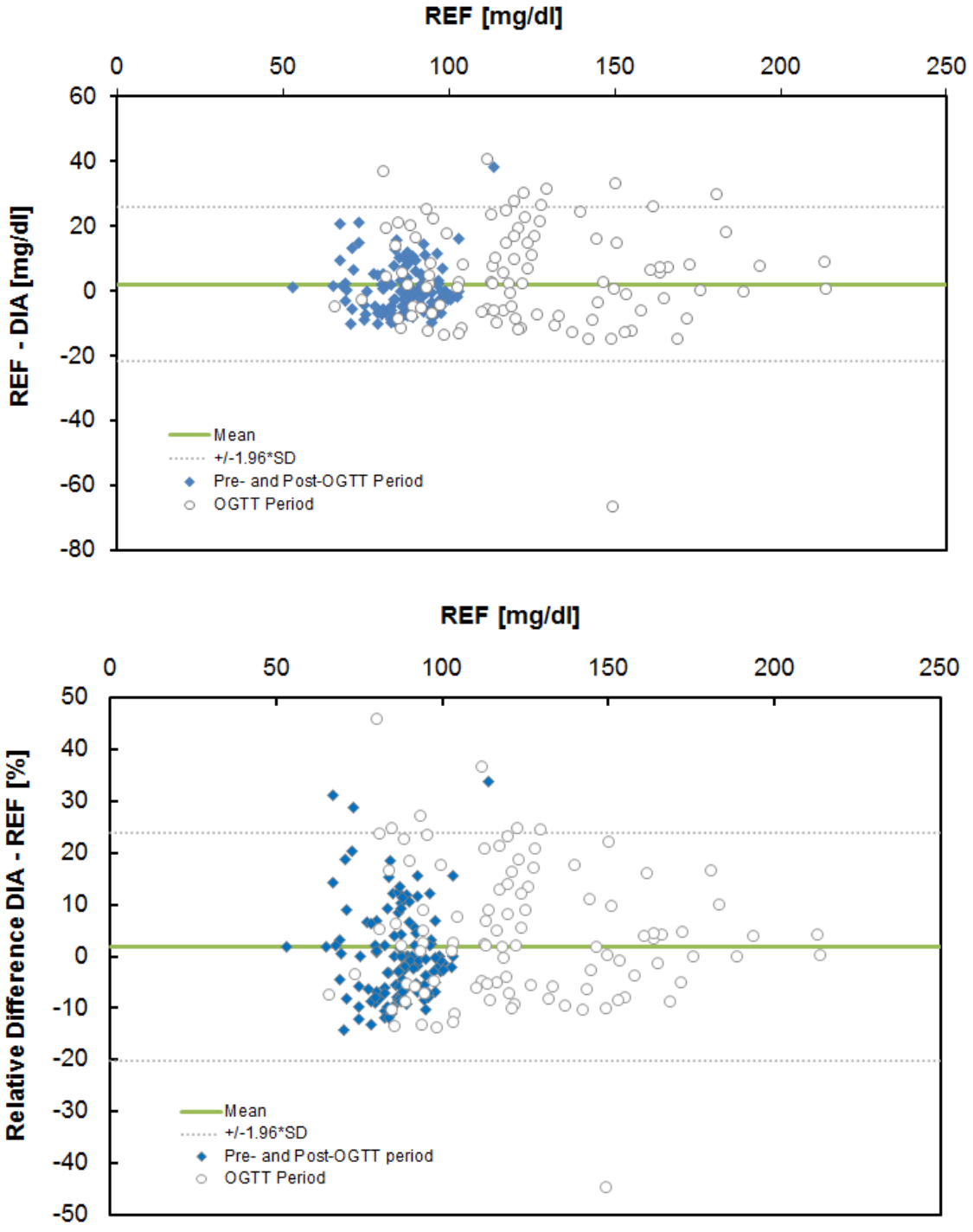

Figure 21: Bland and Altmann plots relating absolute (top) and relative (bottom) differences of transport-time-delay-corrected dialysate (DIA) and reference glucose concentration (REF) data to REF data. Data pairs are shown as full squares during periods of relatively stable glucose concentrations (pre- and post-OGTT period: 0–5 h and 8–12 h, respectively) and as open circles during periods with pronounced glucose excursions (OGTT-period, 5–8 h)

Table 4: Summary of the technical performance evaluation of the 12-h clinical trials performed in eight healthy volunteers undergoing an oral glucose tolerance test (Franz Feichtner et al. 2010)

Subject [#]	CORR [−]	MD [mg/dl]	MAD [mg/dl]	MRD [%]	MARD [%]	%PRESS [%]	ISO MET? [Yes]
1	0.876	6.6±18.2	13.4±13.8	6.7±13.4	11.4±9.5	15.3	21 of 25
2	0.935	5.2±14.8	11.3±10.6	4.0±15.7	11.6±11.0	14.3	21 of 26
3	0.989	−6.5±3.4	6.5±3.4	−6.4±3.1	6.5±3.1	7.1	30 of 30
4	0.936	15.9±10.6	17.0±8.7	16.3±8.9	17.1±7.3	17.8	20 of 27
5	0.995	0.9±4.6	3.6±2.9	0.6±4.3	3.4±2.6	3.9	28 of 28
6	0.984	−6.8±4.6	7.1±4.2	−6.9±4.3	7.2±3.7	8.2	30 of 30
7	0.971	7.5±8.5	8.1±7.9	6.8±7.6	7.6±6.9	11.2	27 of 30
8	0.994	−3.4±3.8	4.0±3.2	−3.6±4.0	4.0±3.5	4.3	30 of 30
ALL	0.960±0.042	2.1±12.1	8.7±8.6	1.9±11.2	8.4±7.7	10.3±5.2	207 of 226

3.2 Clinical performance evaluation

The Clark Error Grid analysis (EGA, (W. L. Clarke et al. 1987)) is a standard clinical evaluation method classifying data pairs of a new glucose sensing method and a reference method into five zones with different levels of clinical acceptability (A: accurate, B: acceptable, C, D and E: not acceptable). EGA analysis revealed that 85.9% (n=195) and 13.7% (n=31) were in zones A (accurate) and B (acceptable), respectively. One glucose sample (0.4%) was found to be in zone D (unacceptable). The EGA plot is depicted in **Figure 22**, left.

Another clinical evaluation method for glucose monitoring systems is the insulin titration error grid analysis (ITEGA). It was developed for intensive care patients undergoing intensive insulin therapy (Martin Ellmerer et al. 2006). Four zones in a scatter-plot represent different degrees of accurate therapeutic treatment assuming the therapy decision was based on the actual glucose measurement (appropriate treatment (a), unacceptable violation (b), major violation (c), life threatening violation (d)).

The ITEGA analysis revealed that 99.1% of all microdialysate (DIA) glucose concentrations would have led to appropriate treatment (zone a), whereas 0.9% (n=2) would have led to an unacceptable violation (zone b) in insulin therapy (compare **Figure 22**, right).

3.3 Safety endpoints

All eight subjects successfully finished the trial without adverse events. For safety reasons, the subject's activated partial thromboplastin time (APTT) - as a measure of systemic heparinisation - was monitored. Three APTT levels were measured per subject and none of them significantly increased during the trial ($p>0.05$).

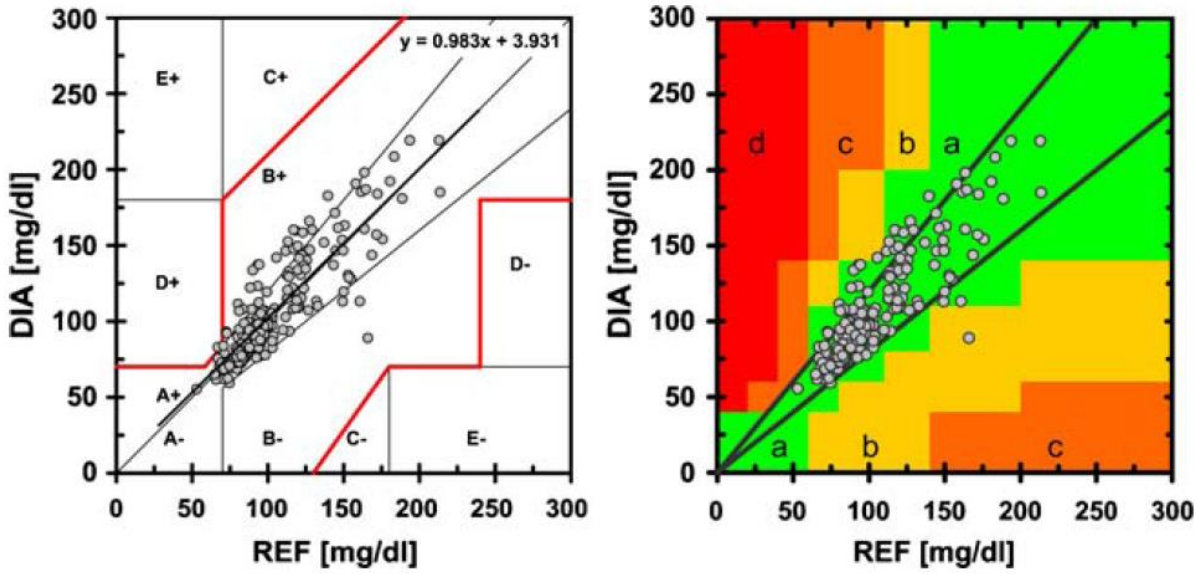

Figure 22: EGA (left) and ITEGA (right) of reference (REF) and non-delay-time corrected microdialysate (DIA) glucose concentration data-pairs (Franz Feichtner et al. 2010)

4. Summary and conclusions[6]

After optimising the operating conditions, a clinical study was performed in eight healthy subjects in order to evaluate the developed body interface technically and clinically. The study was finished successfully in all subjects. A clinical performance evaluation showed that the device is able to deliver glucose information that in 99.6% of all cases was found to be accurate and clinically acceptable.

Also a technical performance evaluation was done considering Bland and Altman analysis and state of the art estimate parameters including absolute and relative differences, mean absolute relative difference, the system error %PRESS and ISO criterion. The latter was met in four out of eight experiments. 78% of all ISO deviations occurred during the OGTT period, thus during times of pronounced glucose changes. No correlation was found between met or unmet ISO criterion and the length or the amplitude of glucose concentration during the OGTT period, but it might be worth considering here that the performed clinical and technical evaluation is based on 15 min-time-integrated glucose concentrations of dialysate samples that were compared to spot measurements of reference blood glucose concentration. Dialysate samples thus represent a 15

[6] Partly taken and adapted from (Franz Feichtner et al. 2010)

min glucose average, which especially in periods with pronounced glucose changes (OGTT period) can result in relatively high discrepancies between glucose concentrations of reference and dialysate blood samples. All other evaluated clinical and technical performance parameters, let us conclude that the developed system stably delivers a continuous dialysate-matrix upon which the estimation of blood glucose concentrations can be performed precisely and highly accurate. However, the system's precision could even be further improved, e.g. by using online glucose sensors instead of offline glucose analysis of time-integrated dialysate samples and by incorporating an online recovery monitor such as suggested by Schaupp et al. (1999) or Yokel et al. (1992).

The current limitations of the system include blood loss, time delay and the lack of an integrated online glucose sensor. The former is inherent and cannot be diminished in our approach, but the blood withdrawal rate of 2 ml/h was chosen according to specifications of the DLC-manufacturer and approved to be safe by intensive care professionals. However, it can be speculated that blood waste might be reduced by further downscaling system dimensions, by re-infusing the analysed blood or by integrating the analytics for other relevant blood gases and metabolites.

The time delay of our system currently is 10.5 min, therefore therapy decision would be based on 'old' glucose data. However, it was shown by the EGA and ITEGA analysis that this time delay would not result in unappropriate treatment. Moreover, the delay time could be improved by increasing perfusate flow or by introducing low-volume online sensors.

The system has currently not been combined with online glucose sensors and thus allows only a limited number of samples to be analysed when used with offline laboratory glucose meters such as the herein used Beckman glucose analyser, which requires at least 10 µl per sample. Integration of low volume online sensors (e.g. 0.5 µl) such as presented by Schaller et al. (Schaller et al. 2009) is feasible, obtaining highly resolved glucose signals using a 5 µl/min perfusate flow and will be investigated in future studies where aspects of longterm in vivo stability will also be addressed.

In summary, a device has successfully been developed and tested that continuously delivers dialysate which is extracted from whole blood outside the human body for the purpose of continuous glucose monitoring. The next step towards an automated continuous glucose monitoring system is to integrate online glucose sensors into the herein presented microdevice.

CHAPTER IV

Comparing the performance results of the developed body interface approach to those obtained with standard subcutaneous microdialysis in a post-hoc analysis of two clinical studies

This chapter reprints the study findings as originally peer-reviewed published by Mader, Feichtner et al. (Julia K Mader et al. 2012)

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International Diabetes Federation

Microdialysis—A versatile technology to perform metabolic monitoring in diabetes and critically ill patients

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ABSTRACT

Continuous subcutaneous glucose monitoring has been tested in type 1 diabetes (T1D). Since in critically ill patients vascular access is granted vascular microdialysis may be preferential. To test this hypothesis comparative accuracy data for microdialysis applied for peripheral venous and subcutaneous glucose monitoring was obtained in experiments in T1D patients.

Twelve T1D patients were investigated for up to 30 h. Extracorporeal vascular (MDv) and subcutaneous microdialysis (MDs) was performed. Microdialysis samples were collected in 15–60 min intervals, analyzed for glucose and calibrated to reference. MDv and MDs glucose levels were compared against reference.

Median absolute relative difference was 14.0 (5.0; 28.0)% (MDv) and 9.2 (4.4; 18.4)% (MDs). Clarke Error Grid analysis showed that 100% (MDv) and 98.8% (MDv) were within zones A and B.

Extracorporeal vascular and standard subcutaneous microdialysis indicated similar performance in T1D. We suggest microdialysis as a versatile technology for metabolite monitoring in subcutaneous tissue and whole blood.

1. Introduction

Over the last years, a number of systems for continuous subcutaneous glucose monitoring (CGM) have been marketed and are routinely used in patients with diabetes to obtain more detailed information about 24-h glucose profiles [1–5]. CGM has proven to be useful in order to improve glycaemic control and reduce the risk of hypoglycaemia in type 1 diabetic patients [3,6]. In pregnant women, CGM has been proven effective in lowering HbA1c without higher rates of hypoglycaemia. Data obtained from continuous glucose monitoring were already used to steer insulin titration algorithms under research conditions [7]. In the future, CGM will be an essential part of an artificial pancreas system for automated closed loop glucose control.

In the hospital, numerous factors influencing glycaemic control and possibly leading to glucose excursions out of the desired range exist, e.g. interventions for which the patient has to remain fasting or medications such as corticosteroids or vasopressors. A reliable CGM could help to observe levels of glycaemia not only at defined time-points but continuously, making it possible for physicians and nursing staff to react before the patient is exposed to severe hypo- or hyperglycaemia [8]. The feasibility of CGM in hospitalized patients has been proven; however there were some substantial deviations

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from the subcutaneous to the reference signal [8–10]. The delay between blood and CGM value results from the physiologic lag time between blood and the interstitial space (maximum 10–15 min) but is controversially discussed [11]. Additionally the individual instrumental lag times have to be considered, which are reported to range from 2 to 10 min depending on the investigated CGM system [12]. In the hospital setting, vascular access is granted in the majority of patients and could be used as an alternative site for continuous glucose monitoring.

Therefore, the aim of the present study was to demonstrate, that using the microdialysis technique venous blood glucose concentration can be monitored both using vascular as well as subcutaneous access.

2. Materials and methods

The current publication analyzes two experiments which were performed in type 1 diabetic patients (MDv: 3 female/1 male, age 31.5 ± 7.7 years, BMI 25.8 ± 6.3 kg/m^2, diabetes duration 11.4 ± 9 years; MDs: 8 male, age 31.5 ± 8.0 years, 24.9 ± 1.7 kg/m^2, diabetes duration 13.6 ± 6.6 years). In four patients vascular extracorporeal microdialysis (MDv group) was performed [13], while in eight standard subcutaneous microdialysis (MDs group) was applied. The subjects were investigated over a period of up to 30 h. To simulate glucose excursions meal protocols with different insulin dosing procedures were applied: in the MDv group the insulin dose was administered intravenously using a glucose control algorithm aiming at a target range of 4.4–6.1 mmol/l, in the MDs group insulin was administered subcutaneously using multiple daily injections (MDI) at the patients' discretion aiming to maintain glycaemia at the patients' individual target range. Both studies were approved by the local ethics committee of Medical University of Graz, Austria, and written informed consent was obtained from all patients before any trial related activities.

2.1. Study period

For both experiments patients arrived at the research facility at 12 am after a fasting period of at least three hours. For reference measurements arterialized venous blood samples were drawn from a venous line inserted into a vein of the forearm which was placed in a thermo-regulated box (50 °C). Reference plasma glucose concentrations were measured twice using a glucose oxidase based method (Beckman Glucose Analyzer 2, Beckman Instruments Inc., Fullerton, CA). Arterialized venous blood glucose readings were used to prospectively calibrate both microdialysis techniques.

In both experiments, patients received four standardized meals: dinner at 6 pm (37 g of carbohydrates (CHO)), a snack at 10 pm (29 g CHO), breakfast at 8 am (36 g CHO) and lunch at 12 am (31 g CHO).

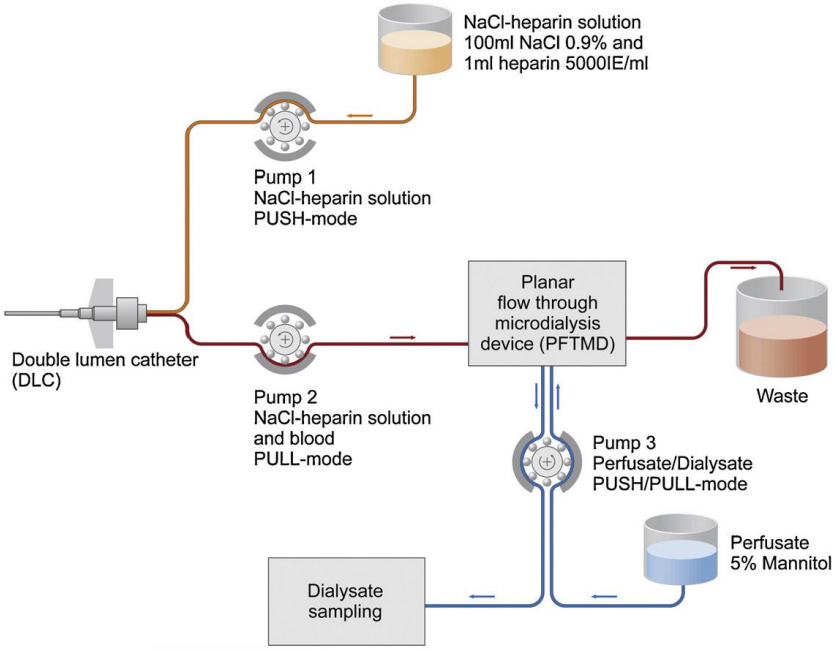

Fig. 1 – Schematic set-up of the extracorporeal vascular microdialysis approach. NaCl–heparin solution is pumped to the double lumen catheter where it mixes with blood. NaCl–heparin/blood mixture is further pumped to the planar flow through microdialyser. 5% mannitol is used as perfusate. Dialysate is collected in a sampling vial for further analysis.

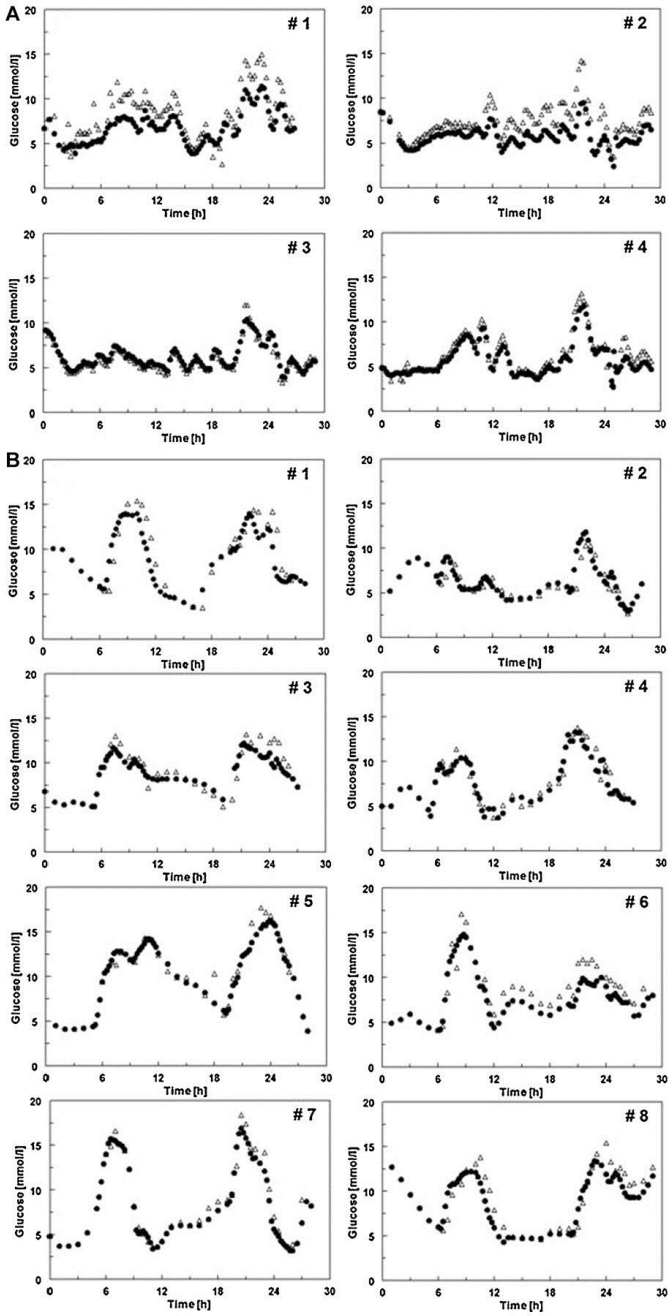

Fig. 2 – (A) Individual glucose profiles for vascular microdialysis experiments. The closed circles indicate reference glucose, the open triangles dialysate glucose. Samples obtained from vascular microdialysis were calibrated to reference at t = 0, subcutaneous samples were calibrated after a run-in period of 6 h. (B) Individual glucose profiles for subcutaneous

Table 1 – Performance of the vascular and subcutaneous microdialysis experiments. Data for MARD as well as CG-EGA are stratified for glucose range.

	MDv (n = 409) Overall performance			MDs (n = 252) Overall performance		
Median difference (mmol/l)	−0.68 [−1.67; 0.04]			0.48 [−0.17; 1.30]		
MAD (mmol/l)	0.77 [0.29; 1.68]			0.79 [0.28; 1.44]		
MARD (%)	14.0 [5.0; 28.0]			9.2 [4.4; 18.4]		
	Hypoglycaemia (≤3.9 mmol/l) n = 9	Euglycaemia (4.0–10 mmol/l) n = 386	Hyperglycaemia (>10 mmol/l) n = 14	Hypoglycaemia (≤3.9 mmol/l) n = 7	Euglycaemia (4.0–10 mmol/l) n = 159	Hyperglycaemia (>10 mmol/l) n = 86
MARD (%)	12.6 [8.3; 14.6]	13.9 [4.9; 28.0]	19.9 [14.1; 29.9]	7.2 [6.1; 14.9]	12.1 [5.3; 20.6]	7.4 [2.4; 12.6]
CG-EGA						
Clinically accurate	100.0%	96.1%	100.0%	100.0%	98.7%	95.3%
Benign errors	0.0%	3.1%	0.0%	0.0%	1.3%	4.7%
Erroneous readings	0.0%	0.8%	0.0%	0.0%	0.0%	0.0%

2.2. *Vascular microdialysis group (MDv)*

For vascular microdialysis peripheral venous blood was continuously withdrawn from a double lumen catheter (DLC; mtb GmbH, Lonsee, Germany) inserted in the forearm of the subject. NaCl–heparin solution was continuously pumped to the tip of the DLC via the outer lumen at a flow rate of 2 ml/h while blood–heparin mixture was continuously withdrawn via the inner lumen of the DLC at a flow rate of 4 ml/h. Venous blood and corresponding blood glucose concentration was thus diluted by 50%. Blood–heparin mixture was then pumped to an extracorporeal membrane based microdialyser (see Fig. 1) to obtain dialysate as previously described [14]. This microdialyser sandwiches a semi-permeable custom-made, flat sheet membrane (10 kDa) between two microfluidic plates in which heparinized blood on the one and dialysate on the other side are perfused (5 μl/min). The instrumental lag-time of the extracorporeal vascular microdialysis approach is 10.5 min as described by Feichtner et al. [14]. Dialysate samples were collected in microtubes exchanged every 15 min throughout the study period. The dialysate samples were immediately analyzed for glucose concentration using a Beckman Glucose Analyzer. Glucose values from vascular microdialysis were calibrated to reference at time zero using a one-point prospective calibration.

2.3. *Subcutaneous microdialysis group (MDs)*

The microdialysis principle has been described in detail elsewhere [15,16]. The microdialysis probe (CMA 60, membrane cut-off: 20 kDa, 30 mm × 0.6 mm) was inserted into subcutaneous adipose tissue of the abdominal region. The microdialysis catheter was continuously perfused with a solution of 5% mannitol at a constant flow rate of 1 μl/min using a portable minipump (CMA 107, CMA Microdialysis AB, Sweden). Dialysate samples were collected in microtubes changed every 30 min over a period of 3 h following meal ingestions and every 60 min during basal periods. Dialysate samples were immediately deep frozen (−80 °C) and later analyzed for glucose concentrations using standard enzymatic assays (Roche Diagnostics, Mannheim, Germany) using a Cobas Mira Analyzer (Hoffmann-La Roche, Basel, Switzerland), and for sodium concentrations using a flame photometer (Instrumentation Laboratory, Vienna, Austria) at the laboratory of Joanneum Research GmbH, Graz, Austria according to GLP (Good Laboratory Practice). Subcutaneous microdialysis samples were then calibrated to blood in a two-step approach as described by Ellmerer et al. [17]: first the ionic reference technique was used to account for sodium recovery, and then a prospective one-point calibration to reference glucose 6 h after catheter insertion was performed. The 6-h run-in period prior to calibration has been reported previously [18] to stabilize perfusion of the probe and thus improve the quality of the subcutaneous signal.

2.4. *Data analysis*

Paired vascular and subcutaneous dialysate values were used to calculate the median difference (dialysate glucose – reference glucose), median absolute difference (MAD, absolute value of difference) and median absolute relative difference (MARD): [(dialysate glucose – reference glucose)/reference glucose] expressed as a percentage.

When using standard subcutaneous microdialysis a fractionized fluid sample is collected over a predefined time period, the reference blood glucose measurements corresponding to a start and an end glucose value of the interstitial samples were averaged and then paired with the according interstitial glucose value.

Data were then graphically displayed in a Bland–Altman plot to indicate the relative differences between the paired glucose readings on the y-axis in relation to the average blood glucose concentration on the x-axis [19,20].

The clinical evaluation of the data obtained from vascular and subcutaneous microdialysis, was performed using the

microdialysis experiments. The closed circles indicate reference glucose, the open triangles dialysate glucose. Samples obtained from vascular microdialysis were calibrated to reference at t = 0, subcutaneous samples were calibrated after a run-in period of 6 h.

Clarke Error Grid [21]. To additionally take the point- and rate-accuracy at different levels of glycaemia (eu-, hyper- and hypoglycaemia) into account, the continuous glucose-error grid analysis (CG-EGA) was utilized [22] (Continuous Glucose Error Grid Analysis software, version 1.0.0.0, Epsilon Group, Charlottesville, VA, USA).

Data fulfilling the criteria for normal distribution are expressed as mean ± SD, otherwise they are expressed as median and interquartile range. All analyses were performed using SPSS 13.0.1 for Windows (SPSS, Chicago, IL, USA) and Microsoft Office Excel 2003 at the Center for Medical Research, Medical University of Graz, Austria.

3. Results and discussion

Study duration was 28.3 ± 1.2 h in the four patients of the MDv group and 28.3 ± 0.5 h in the eight patients of the MDs group. This resulted in 28.3 ± 1.2 evaluable hours of continuous monitoring in the MDv group and 21.7 ± 0.6 h in the MDs group. The shorter duration of the experiment in the MDs group is due to the 6 hour run-in period to allow a stabilization of the microdialysis probe in the subcutaneous adipose tissue.

In total, 409 paired glucose readings for MDv and 252 for MDs were obtained, individual glucose profiles for vascular and subcutaneous microdialysis experiments are displayed in Fig. 2. As expected, glucose levels in the MDv group experiments using an algorithm aiming at a target of 4.4–6.1 mmol/l were lower compared to levels of patients-driven therapy in the MDs group. Median prospectively calibrated glucose concentrations were 6.7 [5.2; 8.3] mmol/l (reference: 5.8 [4.9; 6.9] mmol/l) for the MDv group and 9.0 (6.2; 12.0) mmol/l for the MDs group (reference: 8.8 [6.0; 11.4] mmol/l). Overall and individual levels for median difference, MAD and MARD are shown in Table 1. Results obtained for the accuracy of both microdialysis approaches at different glycaemic ranges (≤3.9 mmol/l, 4.0–10.0 mmol/l, >10.0 mmol/l) showed best performance in the lowest glucose range (Table 1).

The mean glucose differences (2SD) [11.7 (32.4)% for MDv; 2.3 (39.9)% for MDs] are displayed in the Bland–Altman plots (Fig. 3).

Clarke Error Grid analysis (Fig. 4) showed that 100% of the values were within zones A and B (68.1% A, 31.9% B) for MDv. For MDs 98.8% of the values were in zones A and B (76.6% A, 22.2% B), 2 values (0.8%) were located in zone C and 1 value (0.4%) was located in zone D; no value was situated in zone E (0.0%).

According to CG-EGA the performance of subcutaneous and vascular microdialysis was comparable: overall CG-EGA for MDv indicated 99.2% of the readings being clinically accurate or benign errors (96.3% accurate readings, 2.9% benign errors); for MDs 100% of the readings were clinically accurate or benign (97.6% accurate readings, 2.4% benign errors). CG-EGA data stratified for different levels of glycaemia are indicated in Table 1.

4. Discussion

Both vascular and subcutaneous microdialysis signals followed meal-induced glucose excursions similarly and were comparable in terms of accuracy when tested in type 1 diabetic patients over a period of up to 30 h. For both microdialysis techniques the highest levels of accuracy were observed in the clinically crucial hypoglycaemic range according to MARD and CG-EGA. These results, however, have to be interpreted with caution due to the small number of values in the hypoglycaemic range (9 for MDv and 7 for MDs, respectively). One has to keep in mind that in the present studies no lag-time compensation was taken into account. The lag-time between subcutaneous tissue and blood is still a controversial topic in the scientific literature but should not exceed 10 to maximum 15 min [11]. With the sampling intervals chosen for our study the lag-time is therefore expected to have a minor but negligible influence on the data reported.

The performance of subcutaneous microdialysis was in line with data reported in literature [23]. Data from vascular microdialysis at present are still scarce; in contrast to our results obtained from vascular microdialysis, two recently published investigations testing vascular microdialysis [24,25] indicated an inferior performance of a similar approach. This might be due to the fact that in our experiments microdialysis was performed extra-corporeally while both published studies

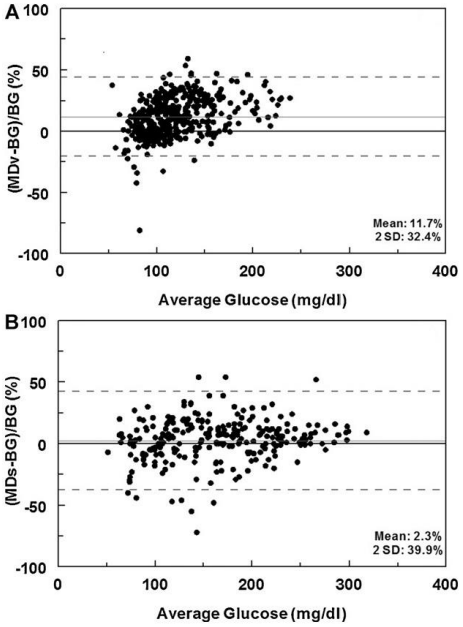

Fig. 3 – Bland–Altman graphs for vascular microdialysis (A) and subcutaneous microdialysis (B). On the x-axis, the reference glucose is displayed, on the y-axis the relative differences between dialysate and reference glucose value are indicated. The solid grey line represents the mean difference; the dashed grey lines represent mean difference 2 times the standard deviation of the differences.

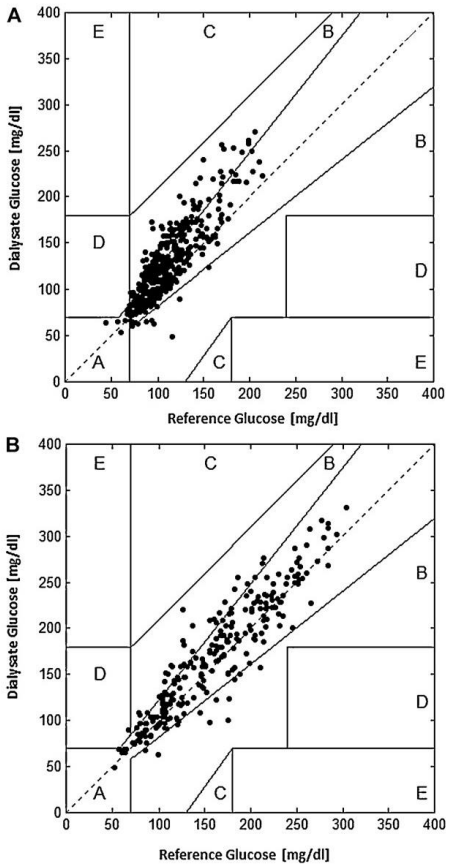

Fig. 4 – Clark error grid for glucose readings obtained using (A) vascular microdialysis (MDv) and (B) subcutaneous microdialysis (MDs) plotted against reference. For MDv 68.1% of the values are in the clinically accurate zone A and 31.9% in the benign error zone B. For MDs 76.6% of the values are within zone A and 22.2% in zone B; 2 values (0.8%) are located in zone C and 1 value (0.4%) is located in zone D.

used intravascular microdialysis approaches. Moreover, in our experiments microdialysis was only performed for a period of up to 30 h whereas both other studies observed patients for a considerably longer period (3 days vs. 5 days). Both, the intravascular location of the microdialysis probe and the longer study period could have led to higher rates of biofouling, blood clotting and permeability problems at the microdialysis membrane. Rooyackers et al. [25] tested intravascular microdialysis in healthy volunteers as well as in critically ill patients in an ICU setting. Unfortunately they did not calibrate the microdialysis signal to blood and a direct comparison with the performance of our extravascular study is therefore not possible.

The aim of the present study was to summarize the data of two individual protocols in order to demonstrate that microdialysis is a versatile technology that can be applied in different settings and at different sampling sites. It is of importance to note, that the aim of this study was not to compare subcutaneous vs. vascular microdialysis. When interpreting the data one has to take into account that the data provided are based on two individual study protocols using an identical study period and meal schedules but different glycaemic target ranges, different sample size ($n = 4$ for MDv vs. $n = 8$ for MDs) and different sampling frequency.

The main field of application for vascular microdialysis is thought to be critically ill patients in an ICU setting, where vascular access is granted. In such a patient population a difference in the accuracy of the glucose signal might be expected due to altered tissue perfusion at states of critical illness favouring the vascular microdialysis approach.

Further experiments testing the vascular microdialysis approach over an extended time period and applied in different patient populations are required to evaluate long-term feasibility of the technique.

Overall we conclude that microdialysis represents an interesting technology that can be applied for glucose monitoring both in vascular as well as in subcutaneous tissue.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgements

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CHAPTER V

Integration of online glucose sensors to form a continuous glucose monitoring (CGM) system and technical and clinical evaluation in a clinical study

1. Introduction

In the first feasibility in-vivo experiment in healthy volunteers, the PFTMD device showed good technical and clinical performance in-vitro and in-vivo in eight healthy subjects but faced several limitations:

- The system lacked the integration of online sensors to continuously monitor glucose concentration profiles.
- The precision of the system could be improved by incorporating the online-monitoring of glucose recovery rates such as suggested by Trajanoski et al. (Trajanoski et al. 1997), Schaupp et al. (Lukas Schaupp et al. 1999) or Yokel et al. (Yokel et al. 1992). With these or similar methods, an online correction of the glucose signal for variable microdialysis efficiencies could be applied.
- The delay time between a valid glucose concentration value in dialysate and venous blood was 10.5 min and was considered needing improvement such that therapy decision would not be based on "outdated" glucose information.

In this chapter the improvement of this device with respect to the before mentioned three limitations is presented. Two previously evaluated online glucose sensors were incorporated and compared (R Schaller et al. 2009; Damm et al. 2007). Both are able to online monitor and correct varying microdialysis efficiencies. Finally the whole device's delay time was more than halved.

A further clinical study was performed in a prolonged duration (30h) in nine healthy subjects to technically and clinically evaluate the microdialysis based device including the two online glucose sensors.

2. Material and methods

2.1 Clinical investigations

A technical and clinical evaluation of the PFTMD in combination with the two online glucose sensors was performed in a 30 h open mono-centre clinical trial in nine healthy, non-diabetic volunteers (all males; age: 27.8 ± 2.3 years; BMI: 22.5 ± 2.4 kg/m²). The study was approved by the local ethical committee and was performed according to Good Clinical Practice (GCP) guidelines at the Clinical Research Centre located at the Medical University of Graz. After

obtaining signed informed consent from each subject, the experiments began according to the study protocol.

In the first subject (#1) no online glucose sensors were used. This trial was used to test new PFTMD-components (i.e. pressure monitoring for heparin infusion monitoring and optical sensing of blood availability for early detection of catheter blockage; see chapter "Continuous blood withdrawal using a double lumen catheter"). However, this subject was included in the technical analysis of the PFTMD device.

The electrochemical sensor was tested in four subjects (#2, 3, 8, 9) as was the spectrometric sensor (subjects #4, 5, 6, 7), with one sensor each. The online glucose sensors were positioned between the PFTMD device and dialysate sampling.

Two subjects had to be rejected from data analysis (#4, 7) due to a permanent technical failure of the continuous blood withdrawal system and due to a permanent fluidic problem of the spectrometric sensor, respectively. Thus, data analysis was performed on the remaining seven analysable subjects.

2.2 Study protocol and sampling of probes

Subjects attended the Research Centre at 11:00 a.m. They could have regular breakfast before 8:00 am. Two venous cannula were inserted into the Vena mediana cubiti of the right and the left arms of the subjects for intermittent reference (CODAN pvb Medical GmbH, Lensahn, Germany) and continuous blood sampling (Double lumen catheter DLC; mtb GmbH, Lonsee, Germany), respectively. After catheter-insertion, the PFTMD was connected to the DLC and continuous blood sampling and extravascular microdialysis was started.

The study protocol started with an initial sampling interval of 60 min. During the 30 h trial period the subjects received four regular meals (dinner, snack, breakfast, lunch) at 5:00 p.m., 9:00 p.m., 8:00 a.m. and 12:00 a.m. to enable the observation of the subject's pre- and postprandial glucose concentration profiles. The sampling interval was reduced to 15 min for three hours starting with each meal ingestion and was increased again to an half-hourly sampling interval for the following hours.

Reference blood samples (REF, 100 µl) were centrifuged and supernatant plasma was collected and immediately analysed for glucose concentration using a Beckman glucose analyser. Microdialysate samples (DIA) were collected for 10 minutes to get enough sample volume for double determinations of glucose concentrations by the Beckman glucose analyser. Thus, DIA

samples reflect 10 min integrated glucose concentrations. REF samples were taken in the middle of each DIA sampling period. Additional blood samples were taken for activated partial thromboplastin time (APTT) and haemogram determination each 5 hours to verify the absence of any significant patient heparinisation.

2.3 Spectrometric glucose sensor

The infrared spectrometric glucose sensor consists of an IR200 mini-spectrometer (Thermo Electron Corporation, Madison, USA) equipped with a room temperature DTGS-detector (deuterated triglycinsulfate) and a custom-made micro-cell with CaF_2 windows (optical sample pathlength 32 µm, internal volume 0.6 µl; spectral resolution of the spectra: 16 cm^{-1}; for the fluidic system with flow-through cell, see **Figure 23**). The spectrometer cell is alternately rinsed with dialysate (10.5 min) and Ringer's solution (3.5 min) for glucose and spectral background measurements using a two-way magnetic switch valve (Takasago Electric, Inc., Nagoya, Japan). Dialysate and reference absorbance spectra are recorded shortly after another, thus spectrometer drift or changes in atmospheric absorptions within the spectrometer can be minimised and the quality of spectra is high. A laptop computer running a MATLAB program was used for data acquisition and interpretation using quantitative multivariate analysis of the dialysate absorbance spectra. Details of the MATLAB code have been presented elsewhere (H. M. Heise, Damm, Manfred Bodenlenz, et al. 2007a). For quantitative glucose determination, spectral features within the so-called fingerprint interval of the mid-infrared spectral range of 1580 – 950 cm^{-1} have been exploited using classical least-squares fitting for the modelling of the experimental spectra, providing on average a sensor readout each five minutes.

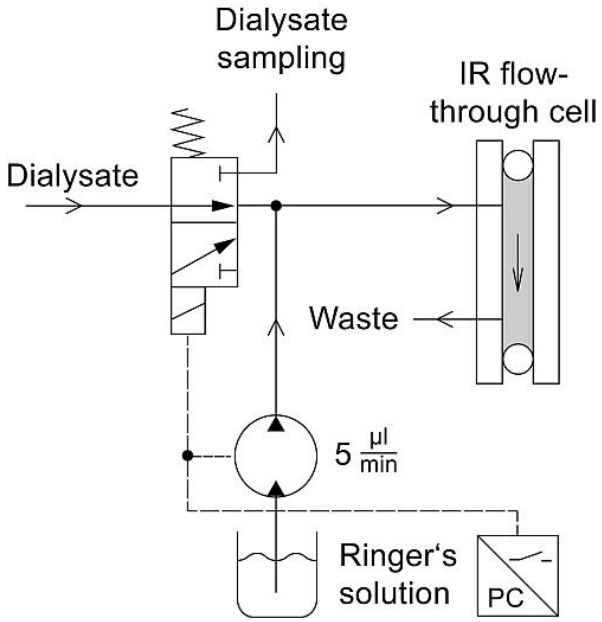

Figure 23: Schematic illustration of the fluidics including the spectrometric glucose sensor cell that is alternately rinsed with whole blood microdialysate and Ringer's solution for the recording of dialysate and reference absorbance spectra. The cell was placed within the sample compartment of the FTIR-spectrometer

2.4 Electrochemical glucose sensor

The electrochemical thick film glucose biosensor is a screen-printed amperometric sensor layered with polycarbamoyl sulfonate (PCS) immobilised glucose oxidase (GOD; (Kotte et al. 1995)). The sensor was developed by Senslab during the term of CLINICIP. Details regarding sensor cell preparation have been presented elsewhere (Roland Schaller, Franz Feichtner, Hans Köhler, Manfred Bodenlenz, Johannes Plank, A. Wutte, Julia K Mader, Martin Ellmerer, Robert Hellmich, et al. 2009; Gruendig et al. 2006). On top of the GOD-PCS matrix layer a diffusion and protection layer made of siloprene is deposited that controls and limits the rate of chemical reaction. It specifically forwards oxygen by diffusion and constrains glucose diffusion so that the ratio between oxygen and glucose is between 10 and 50 ensuring the linear dynamic range of the sensor, which is between 9 mg/dl and 360 mg/dl glucose in vitro. The sensor is provided in a micro-structured planar flow-through cell, in which an elliptic groove is embossed around a central exalted area (see **Figure 24**). The dialysate sample flows along the elliptic flow channels, the gap between the measuring window of the sensor and the exalted area of the flow-through cell (with a total internal volume of ~0.18 µl), which is filled by capillary forces. In this way, most of micro-air bubbles are trapped in the elliptic flow channels and do not affect the sensitive area of the indicating window.

The current signal from the amperometric sensor (1-100 nA) is 12-bit A/D converted, recorded, pre-processed and stored onto a non-volatile data memory in 1-min intervals with a data logger (developed by Disetronic, Burgdorf, Switzerland). Data can be transmitted to a PC for storage and further data analysis via an infrared interface.

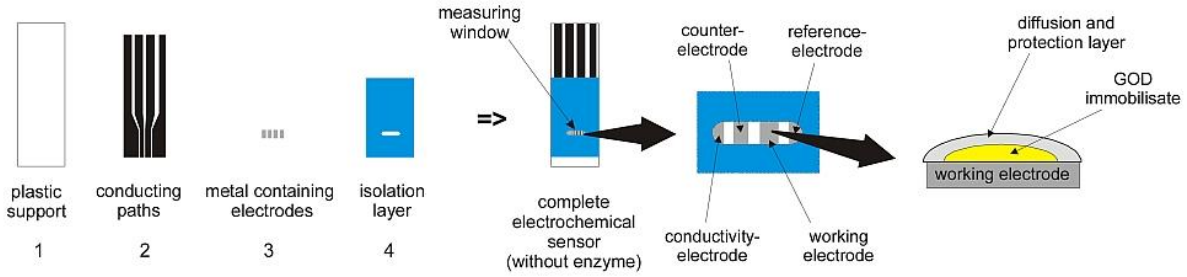

Figure 24: The Senslab sensor (Gruendig et al. 2006) consists of a plastic support (1) on which four conducting paths made of polymeric carbon paste are printed (2, black). In the area of the measuring window, three of the four conducting paths are overprinted by a platinised carbon paste forming working-, counter- and conductivity-electrodes. The reference electrode – the fourth electrode – is overprinted by a silver/silver chloride paste. In a last screen-printing step an isolating (dielectric) paste (4) is overprinted defining the measuring window of the sensor

2.5 Monitoring of dialysis efficiency (recovery monitoring)

In subjects where the spectrometric online glucose meter was applied, ELO-MEL isotonic solution (Fresenius Kabi, Graz, Austria) was used as perfusate. It contains acetate at a concentration of 45 mM. As the physiological concentrations of acetate found in human body fluids are much lower, the loss of this marker substance by diffusion into the heparinised blood inside the PFTMD device can be used to quantify the transport processes across the microdialysis membrane. The ISAS glucose sensor is actually a multi-analyte device with the option also to measure simultaneously the acetate concentration in dialysate by infrared spectrometry. Thus, it allows the simultaneous determination of the dialysis recovery rate, thereby giving accurate dialysate glucose concentration profiles even under varying dialysis efficiencies (H. M. Heise, Damm, Venkata R. Kondepati, et al. 2007)).

In subjects where Senslab sensors were used, ion-free 5% Mannitol solution (Fresenius Kabi, Graz, Austria) was used as perfusate. As the extracellular ion-composition can be regarded as stable in time, changes in the dialysate's ion concentration levels thus reflect changes in the microdialysis recovery. Together with the counter electrode the conductivity electrode of

Senslab's amperometric glucose sensor realises an impedance measurement of the dialysis fluid. The electrical impedance is a measure of the dialysate's ionic composition allowing also an online recovery determination. Thereby, a method for online compensation of varying dialysis efficiency can be established (Lukas Schaupp et al. 1999).

2.6 Calibration procedure

For the technical evaluation of the PFTMD device in combination with the continuous blood sampling system, DIA data have been prospectively 1-point calibrated to reference blood glucose concentration using the first valid data pair.

The amperometric glucose sensor's current signal was averaged on a five minute base. After a 3 hour run in period both sensor signals were prospectively 1-point calibrated to dialysate glucose concentration (DIA) for a technical performance evaluation and to reference blood glucose concentration (REF) for a clinical evaluation. In vitro determinations of the sensors' accuracies, precisions and long-term stabilities have been presented before (Roland Schaller, Franz Feichtner, Hans Köhler, Manfred Bodenlenz, Johannes Plank, A. Wutte, Julia K Mader, Martin Ellmerer, Robert Hellmich, et al. 2009; H. M. Heise, Damm, Venkata R. Kondepati, et al. 2007; Damm et al. 2007).

2.7 Data analysis

The technical validation of the whole device was again performed in accordance to Wentholt et al. (Wentholt et al. 2008) using the same evaluation parameters as in the first clinical trial. The technical performance evaluation for the PFTMD device alone was done on paired glucose readings of delay time corrected, prospectively 1-point calibrated DIA data and reference blood samples (REF). The technical performance evaluation for the glucose sensors alone, online glucose data have been technically assessed using the same parameters, but sensor data were prospectively 1-point calibrated and compared to DIA instead of REF data as described before.

The clinical performance evaluation was done on paired glucose readings of both calibrated sensor systems and reference blood samples, respectively, using again EGA and ITEGA. Here the combination of PFTMD system and online glucose sensors were clinically evaluated. No delay time correction was applied, as it would not be possible in a clinical setting. Data analysis was performed using Microsoft Excel (Microsoft, Inc., Redmond, WA, USA).

3. Results

From seven analysable subjects 485 REF samples were taken (<80 mg/dl: 5; 80 - 110 mg/dl: 272; >110 mg/dl: 204), 12 REF samples were missing or erroneous (2.4%). With the PFTMD device 472 DIA samples were obtained, giving 25 erroneous or missing DIA samples (5.0%), of which most resulted from occlusions at the tip of the double lumen catheter.

3.1 Results of the technical performance evaluation

Applying cross-correlation analysis on REF and DIA data revealed that the delay time of the PFTMD system averaged 3.7min. Evaluating prospectively one-point calibrated glucose concentrations of PFTMD device against whole blood glucose concentrations showed that the mean coefficient of correlation (R) was 0.900 ± 0.10. Mean difference (MD) was found to be 6.2 ± 17.7 mg/dl. Mean absolute (MAD), mean relative (MRD) and mean absolute relative differences (MARD) were 13.8 ± 12.6 mg/dl, 4.7 ± 14.2 % and 11.8 ± 9.2 %, respectively. %PRESS averaged 13.6 ± 6.6 % and ISO criteria were met in 79.7 % of all cases.

Four and two subjects were tested using the combination of PFTMD device and amperometric or spectrometric glucose sensor, respectively. Corresponding time profiles of the online glucose concentration profiles are shown in **Figure 26** and **Figure 27**, respectively.

Technical performance analysis was elaborated on prospectively one-point calibrated sensor data and DIA data, which represent the glucose concentration of the matrix that is presented to the sensors.

With respect to the amperometric glucose sensor, R was 0.498 ± 0.44. MD, MAD, MRD and MARD averaged -5.8 ± 12.9 mg/dl, 9.5 ± 10.4 mg/dl, -9.8 ± 23.9 % and 17.9 ± 18.6 %, respectively. %PRESS was 28.3 ± 4.7 and ISO criterion was met in 81.4 % of all data pairs.

With respect to the spectrometric glucose sensor, R was 0.944 ± 0.03. MD, MAD, MRD and MARD averaged -1.0 ± 6.4 mg/dl, 5.1 ± 4.0 mg/dl, -1.9 ± 12.0 % and 9.7 ± 7.3 %, respectively. %PRESS was 11.8 ± 0.2 and ISO criterion was met in 97.7 % of all data pairs. All details of this technical performance assessment are summarised in Table 5.

3.2 Results of the clinical performance evaluation

Figure 28 presents the results of the Clark Error Grid Analysis (top) and the Insulin Titration Error Grid Analysis (bottom) of the amperometric (left) and the spectrometric (right) glucose sensor of

the investigated subjects. EGA analysis of the spectrometric sensor revealed that all data points are in the A and B zones (A: 91.5%, B: 8.5%, n = 130), whereas the EGA analysis for the amperometric glucose sensor revealed that only 65.7% are in zone A, 31.7% in zone B, 1.9% in zone C and 2.6% in zone D (n=268).

Applying the insulin titration grid analysis revealed that 100% of all spectrometric glucose values would have led to an acceptable treatment if these glucose concentrations had been used as a basis for insulin titration decision in intensive care patients, whereas using the amperometric glucose sensor 90.7% of all glucose values would have led to acceptable treatment, 7.8% to an unacceptable violation and 1.5% to a major violation.

3.3 Safety endpoints

No trial related adverse events occurred. Nonetheless, severe problems with the heparin administration pump were observed in one subject. The pump head squeezed the heparin administration tubing and caused a leakage. Thus heparin was not administered correctly to the DLC and continuously withdrawn blood could not be properly anticoagulated and numerous catheter occlusions followed. However, the subject was not at risk at any time, but the experiment data were excluded from data analysis.

To verify the absence of any significant, unintended subject heparinisation was monitored by determining the activated partial thromboplastin time (APTT) as a measure of systemic heparinisation in a five hourly interval. None of the APTT levels significantly increased during the trial.

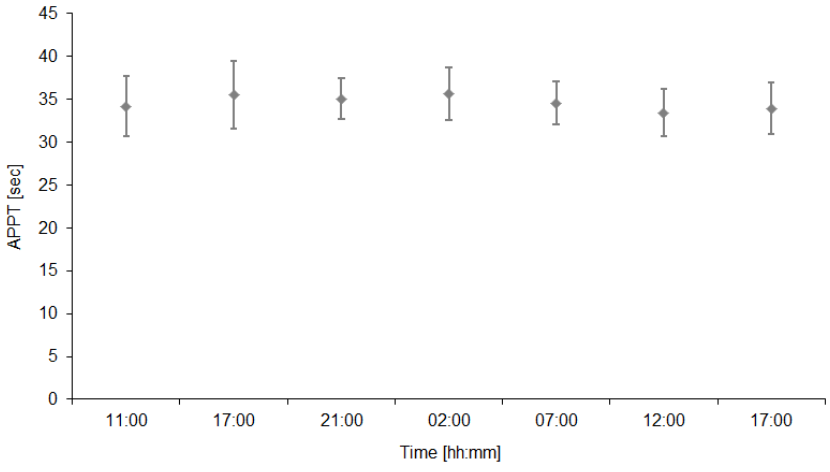

Figure 25: Time course of the subject's APTT levels, which were monitored during the clinical trial to verify the absence of any unintended heparin infusion via the continuous blood withdrawal system.

Table 5: Technical performance evaluation summary for the PFTMD device and the online glucose sensors: PFTMD evaluation was performed on prospectively one-point calibrated, transport-time-delay-corrected blood microdialysate samples against reference blood samples. Sensor evaluation was performed on prospectively one-point calibrated sensor signals against DIA samples. Evaluation parameters include the Pearson's coefficient of correlation (R), mean difference (MD), mean absolute difference (MAD), mean relative difference (MRD), mean absolute relative difference (MARD), %PRESS and ISO-criteria (ISO MET?) as suggested by Wentholt et al. (Wentholt et al. 2008). Evaluation parameters are given as means ± standard deviation for each subject and for all subjects in summary.

SYSTEM	SUBJECT	R	MD	MAD	MRD	MARD	% PRESS	ISO MET?
	[#]	[-]	[mg/dl]	[mg/dl]	[%]	[%]	[%]	[%]
PFTMD only: DIA vs. REF	1	0.934	-0.0 ± 9.2	6.9 ± 6.1	-0.1 ± 9.1	6.8 ± 6.0	8.3	95.3
	2	0.697	-9.9 ± 10.3	11.2 ± 8.9	-9.4 ± 8.7	10.7 ± 7.0	13.7	90.3
	3	0.936	-4.2 ± 7.9	6.9 ± 5.7	-3.8 ± 7.9	6.8 ± 5.6	8.4	95.5
	5	0.895	17.2 ± 15.5	20.5 ± 10.5	14.5 ± 13.6	17.9 ± 8.6	20	58.0
	6	0.966	29.7 ± 18.1	30.6 ± 16.4	22.4 ± 9.6	22.9 ± 8.4	24.9	34.9
	8	0.947	12.5 ± 10.7	14.3 ± 8.1	11.2 ± 10.0	12.8 ± 7.8	12.8	85.9
	9	0.968	-2.3 ± 9.2	6.6 ± 6.8	-2.2 ± 6.0	5.0 ± 3.8	7.2	98.5
	ALL	0.900 ± 0.10	6.2 ± 17.7	13.8 ± 12.6	4.7 ± 14.2	11.8 ± 9.2	13.6 ± 6.6	79.7
Amperometric sensor vs. DIA	2	0.192	-1.3 ± 13.0	6.5 ± 11.4	-2.2 ± 29.8	14.6 ± 26.1	29.9	93.8
	3	0.043	-3.8 ± 17.3	13.1 ± 11.8	-5.9 ± 32.1	24.6 ± 21.3	34.1	68.8
	8	0.825	-9.6 ± 9.2	9.9 ± 9.0	-16.6 ± 11.7	17.2 ± 10.8	25.6	82.3
	9	0.930	-8.6 ± 8.9	9.0 ± 8.5	-14.4 ± 11.5	15.4 ± 10.0	23.6	80.9
	ALL	0.498 ± 0.44	-5.8 ± 12.9	9.6 ± 10.4	-9.8 ± 23.9	17.9 ± 18.6	28.3 ± 4.7	81.4
Spectroscopic sensor vs. DIA	5	0.922	-4.8 ± 4.3	5.4 ± 3.5	-8.9 ± 7.9	10.1 ± 6.1	11.9	98.5
	6	0.965	3.1 ± 5.7	4.7 ± 4.5	5.8 ± 11.1	9.2 ± 8.5	11.6	96.7
	ALL	0.944 ± 0.03	-1.0 ± 6.4	5.1 ± 4.0	-1.9 ± 12.0	9.7 ± 7.3	11.8 ± 0.2	97.7

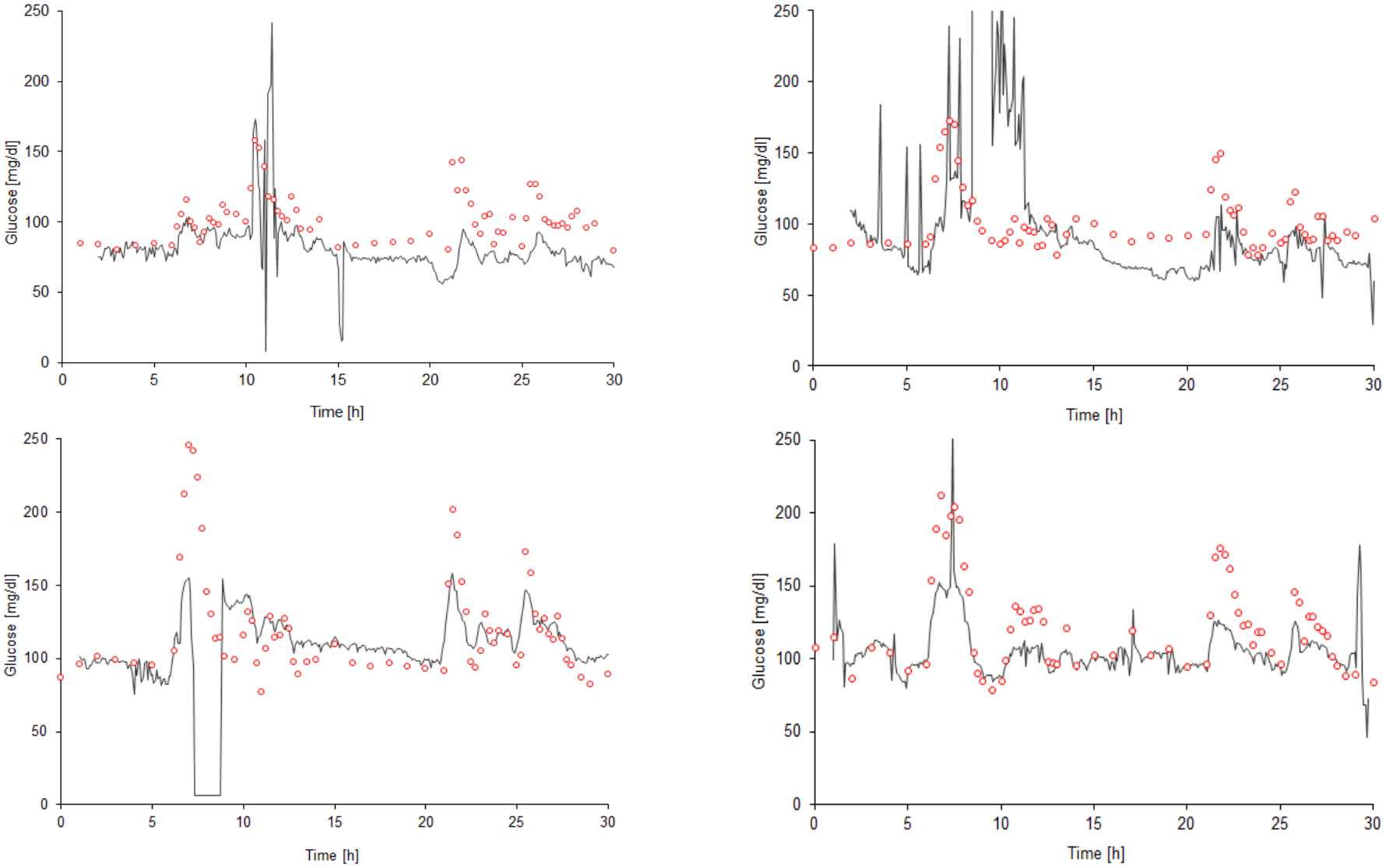

Figure 26: Glucose concentration time profiles of prospectively one-point calibrated amperometric glucose sensor signals (solid lines) vs. reference blood glucose concentrations (open circles) of subjects 2 (top left), subject 3 (top right), subject 8 (bottom left) and subject 9 (bottom right)

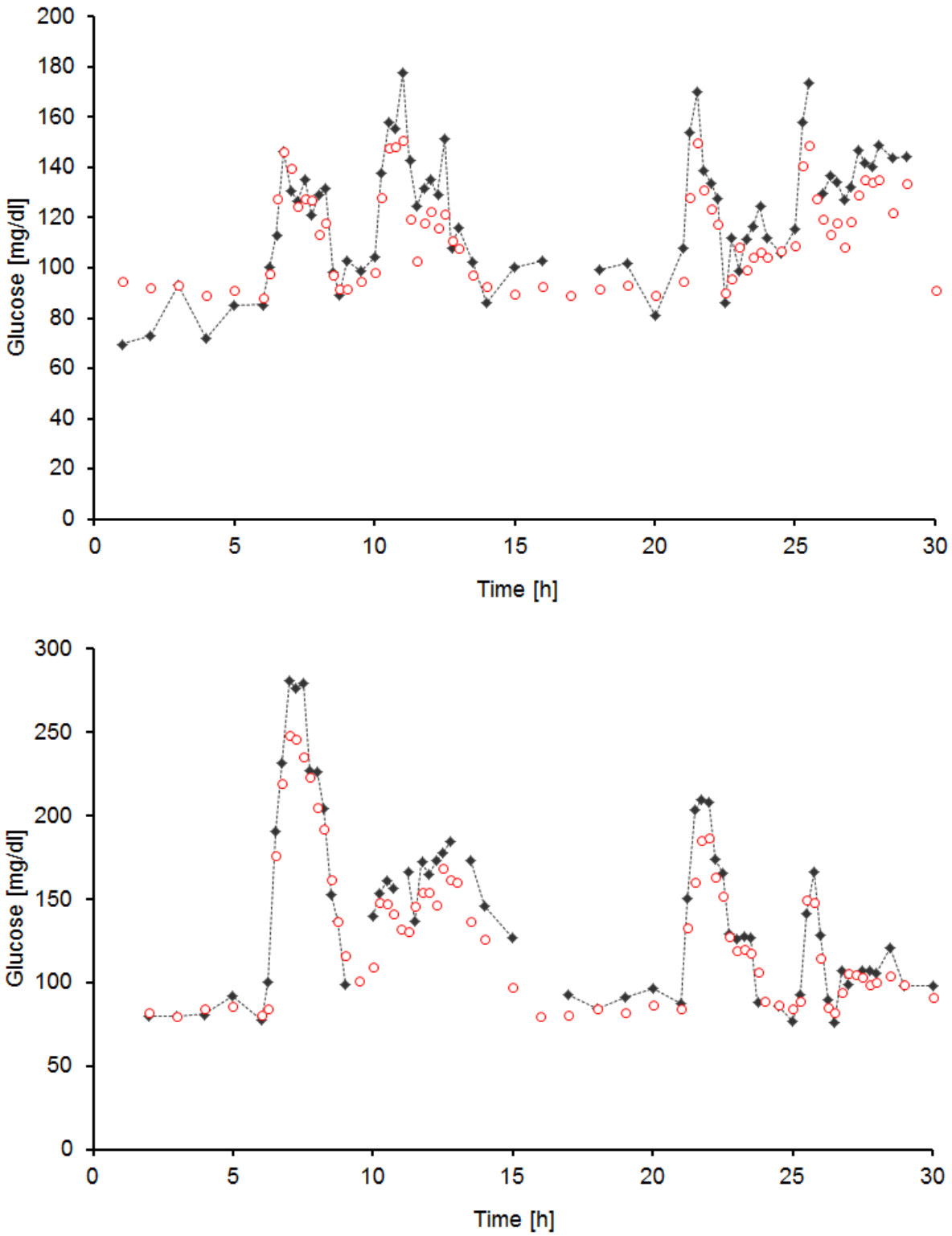

Figure 27: Glucose concentration time profiles of prospectively one-point calibrated spectrometric glucose sensor signals (full diamonds) vs. reference blood glucose concentrations (open circles) of subject 5 (top) and 6 (bottom)

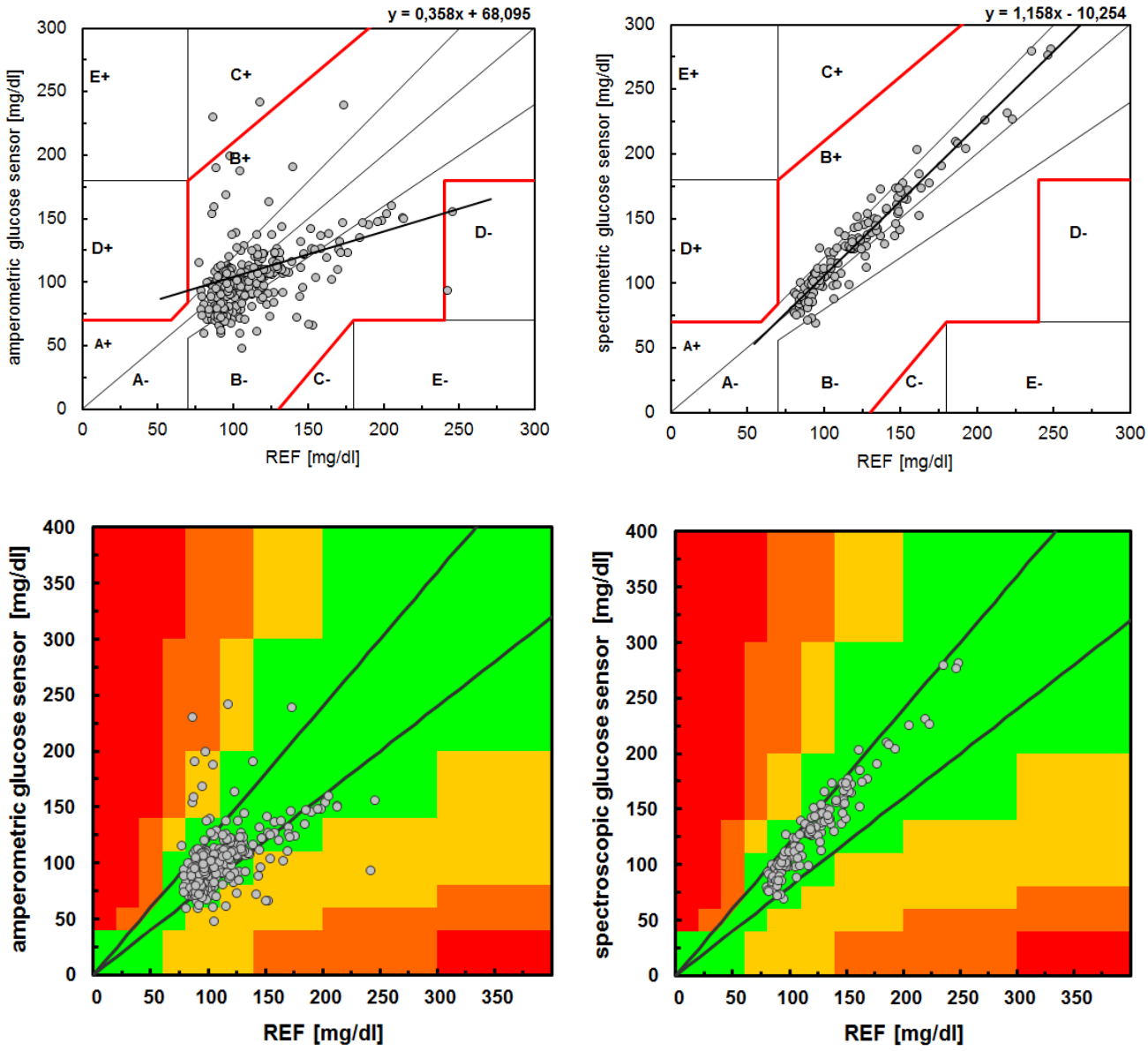

Figure 28: Clark Error Grid Analysis (EGA, top) and Insulin Titration Error Grid Analysis (ITEGA, bottom) comparing reference blood glucose concentrations (REF) and prospectively one-point calibrated amperometric (left) and spectrometric (right) online glucose sensor values derived from measuring whole blood microdialysates. Four subjects have been tested with an amperometric glucose sensor each and two subjects have been tested using the spectrometric system

4. Summary and conclusions

In this study two previously tested systems were combined to form an online glycaemic monitoring device: An amperometric (Gruendig et al. 2006) and an infrared spectrometric (H. M. Heise et al. 2008) online glucose sensor were respectively incorporated into a device that continuously extracted a protein-free biofluid from whole blood based on microdialysis (Franz

Feichtner et al. 2010). Three major challenges for the previously presented microdialysis system existed as outlined in the introduction. Those challenges could be met:

- two online glucose sensors were incorporated into the device
- methods for the online compensation of varying dialysis efficiencies were incorporated into these sensors and
- the delay time was reduced from 10.5 min to 3.5 min, by downscaling system dimensions

Considering the results of the in vivo experiments, it can be reported that the excellent technical performance of the PFTMD device could be repeated in a prolonged study setting, compared to the first clinical study (see chapter III). Erroneous DIA sample taking was slightly decreased compared to the previously presented 12 h experiment (5.0% vs. 5.4%), despite the prolonged study duration.

With respect to the amperometric sensor a moderate performance was achieved based on the technical and clinical performance evaluation. The amperometric sensor experienced difficulties in following high dynamic glucose excursions. Maybe the amperometric sensor performance could be improved by implementing a multiple calibration strategy, e.g. applying non-linear regression. The amperometric sensors might also perform better if it was calibrated in vitro before in vivo application.

For sure, a one point prospective calibration strategy is challenging for glucose sensors in general, especially in terms of drift and loss of dynamic. On the other hand a one point prospective calibration strategy is most likely to be realisable in an ICU setting, as it needs the least time and work effort.

It was therefore decided beforehand to assess both sensors identically and to perform a prospective one-point calibration three hours after the start of each investigation.

The spectrometric glucose sensor can be regarded as excellent considering the technical (MD, MAD, MRD, MARD) as well as the clinical (EGA, ITEGA) performance evaluation. However, only two subjects were analysed using this sensor approach.

What regards sensor size, it has to be noted, that the amperometric sensor is a compact handheld device, whereas the spectrometric glucose sensor is a bedside device.

To conclude, a blood microdialysis device and online glucose sensors were successfully combined to provide a glycaemic monitoring device, which is developed for and intended to work in

intensive care patients. The next step towards a closed loop system is the integration of a glucose control algorithm, which is described in chapter VII.

CHAPTER VI

Comparing the performance results of the developed extravascular microdialysis approach to standard subcutaneous microdialysis in a post-hoc analysis of two clinical studies including online glucose sensors

This chapter reprints study findings that were published as a peer-reviewed article by Heise, Kondepati, Damm, Licht, **Feichtner**, et al. (H. M. Heise et al. 2008) and as a book chapter by Heise, Damm, Kondepati, Mader, **Feichtner** and Ellmerer (Heise et al. 2010)

Microdialysis based monitoring of subcutaneous interstitial and venous blood glucose in type 1 diabetic subjects by mid-infrared spectrometry for intensive insulin therapy

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ABSTRACT

Implementing strict glycemic control can reduce the risk of serious complications in both diabetic and critically ill patients. For this purpose, many different blood glucose monitoring techniques and insulin infusion strategies have been tested towards the realization of an artificial pancreas under closed loop control. In contrast to competing subcutaneously implanted electrochemical biosensors, microdialysis based systems for sampling body fluids from either the interstitial adipose tissue compartment or from venous blood have been developed, which allow an ex-vivo glucose monitoring by mid-infrared spectrometry. For the first option, a commercially available, subcutaneously inserted CMA 60 microdialysis catheter has been used routinely. The vascular body interface includes a double-lumen venous catheter in combination with whole blood dilution using a heparin solution. The diluted whole blood is transported to a flow-through dialysis cell, where the harvesting of analytes across the microdialysis membrane takes place at high recovery rates. The dialysate is continuously transported to the IR-sensor. Ex-vivo measurements were conducted on type-1 diabetic subjects lasting up to 28 hours. Experiments have shown excellent agreement between the sensor readout and the reference blood glucose concentration values. The simultaneous assessment of dialysis recovery rates renders a reliable quantification of whole blood concentrations of glucose and metabolites (urea, lactate etc) after taking blood dilution into account. Our results from transmission spectrometry indicate, that the developed bed-side device enables reliable long-term glucose monitoring with reagent- and calibration-free operation.

Keywords: Infrared spectroscopy, hyperglycemia, continuous glucose monitoring, interstitial fluid dialysate, whole blood dialysate

1. INTRODUCTION

Diabetes mellitus with hyperglycemia over extended periods is a growing health problem. Hyperglycemia is also found in most critically ill patients, even those without a clinical history of diabetes.[1-4] Tight glycemic control in diabetic patients can dramatically delay the onset of serious complications. Similarly, strict glycemic control by lowering the blood glucose levels to 80 – 110 mg/dL, reduces intensive care unit (ICU) mortality, morbidity and duration of hospital stay.[4-10] An indispensible tool for improving glycemic control in diabetic and critically ill patients is reliable blood glucose measurement technology. The different glucose monitoring technologies available – including self-monitoring and continuous blood glucose monitoring devices – and their state-of-the-art were recently reviewed by us.[11] Electrochemical sensing by use of glucose oxidase or glucose dehydrogenase has been the key technology applied for intermittent or continuous blood glucose monitoring in the past.

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Continuous glucose monitoring approaches can be broadly classified into three types: implantable needle-type sensors, minimally invasive sensors based on microdialysis or transdermal fluid transport, and noninvasive optical spectroscopic sensors. While the implantable sensors are limited due to progressive sensor fouling, bio-incompatibility, frequent re-calibrations and insufficient sensor life time, non-invasive sensors are not yet in reach, for example, due to high background absorption of water, instrumental drifts, lack of sensitivity, overfitting during calibration, poor precision and the effect of blood flow on the measurements.[11] Minimally invasive sensors based on measuring the concentration of glucose in interstitial or transdermal fluids are a promising alternative technology.

The clinical application of microdialysis has become feasible with the introduction of commercially available microdialysis catheters. These catheters mimic blood capillaries, where the analyte transport is based on diffusion through a semipermeable membrane. In most cases, microdialysis probes have been placed subcutaneously to allow compounds of low relative molecular mass, such as glucose from the interstitial fluid, to penetrate the dialysis membrane into the dialysate. Using a continuous or discontinuous flow of perfusate, the harvested dialysate can be transported to an extracorporeal measuring device. However, the time lag between sampling and signal detection, and the tissue reaction after the insertion of a microdialysis catheter into the subcutaneous adipose tissue are the major limitations. Alternatively, the subcutaneous body interface can be replaced by a vascular body interface that includes a double-lumen venous catheter in combination with whole blood dilution using a heparin solution. The diluted whole blood can be transported to a flow-through dialysis cell, where the harvesting of analytes across a flat microdialysis membrane takes place at high recovery rates. The dialysate again can be transported to an extra-corporeal sensor.

In this paper, we present the results from both subcutaneous and vascular body interface approaches applied in combination with mid-infrared (MIR) spectrometry. The developed MIR spectrometric sensor is fully automated including its microfluidics for sample transfer. It quantitatively determines the glucose concentrations in microliter volumes of dialysates using transmission spectroscopy. The automation allows sample transfer to the measurement micro-cell housed within the spectrometer sample compartment and repeat spectral background measurements for reliable continuous long-term application. Furthermore, it also allows automatic air bubble detection and removal, simultaneous microdialysis recovery rate estimation besides further multi-component assay capability for other important metabolites. The proof of principle for this system,[12, 13] its in-vitro performance[14] and results from continuous subcutaneous interstitial glucose monitoring in healthy individuals using microdialysis has already been reported by us.[15, 16] Now we discuss its long-term applicability in type 1 diabetic subjects in combination with both subcutaneous and vascular body interfaces.

2. EXPERIMENTAL

2.1 Instrumentation for glucose monitoring in subcutaneous interstitial fluid dialysates

Compared to our first prototype,[15] our fluidic system has been simplified avoiding expensive computer-controlled multiport valves. The schematic set-up for glucose monitoring in subcutaneous interstitial fluid dialysates is shown in Figure 1. It requires an implantable microdialysis catheter CMA 60 from CMA Microdialysis AB (Solna, Sweden), a custom-made patient wearable mini-fluidic system that consists of two mini-peristaltic pumps (one for transporting perfusate to the catheter at 1 µl/min, and another for advancing Ringer's solution at 5 µl/min for sample transport to the spectrometer and back ground measurements) and a two-way magnetic switch valve (Takasago Electric, Inc., Nagoya, Japan), ancillary electronics for controlling the fluid transport, a laptop computer and an IR200 mini-spectrometer (Thermo Electron Corporation, Madison, U.S.A) equipped with a room temperature DTGS detector and a Peltier cooled transmission micro-cell. The magnetic valve consists of two electronically controlled clamps for holding and controlling the fluid transport within the fluidic (Tygon) tubing. The Tygon tubes from the peristaltic pumps were connected to the magnetic valves for electronic control, thus directing the dialysate and reference solutions to be transported alternatively to the spectrometer or waste (see Figure 2).

The dialysate from the subcutaneous tissue was transported to the spectrometer via the N-tubing piece controlled by the 2-way clamping valve. The transport solution flow as controlled by its mini-peristaltic pump was synchronized with the clamp valve switching. By using the clamping valve, the flow of the dialysate and transport solution into the cuvette is controlled. When the clamping valve is switched to open mode connecting to the cuvette line, the controllable mini-peristaltic pump halts its function so that the dialysate is pumped into the tube connected to the cuvette (length 125 cm, internal volume ~ 16 µl) for a duration of six minutes. The clamping valve will be turned to the

close mode and the mini-peristaltic pump starts its operation for advancing the dialysate to the spectrometer flow-through cell over a duration of two minutes providing a volume of ~ 10 µl, enough for cleansing the cell and preparing for spectral background measurements with the Ringer's solution filled cuvette. During this period two background spectra over 30 s of interferogram accumulation from the previous Ringer's solution injection were recorded, which were averaged for further processing. When the clamp valve is in the closed mode, the dialysate flow is directed towards the waste. After this, the transport flow is stopped, while the clamp valve is switched to enable for a repeat dialysate loading into the cuvette tubing line. This cycle of eight minutes is repeated for semi-continuous glucose measurements. Sensor readings were thus realised for every 10 minutes. Depending on the flow rate and the internal volume of the tubing connecting between N-piece and flow-through cuvette, the sample plug optimum and that for the background measurement will be adjusted by the software by sensing the rise in absorption when the first part of the dialysate sample plug enters the cuvette and by slightly changing the duration of the peristaltic pump being switched on.

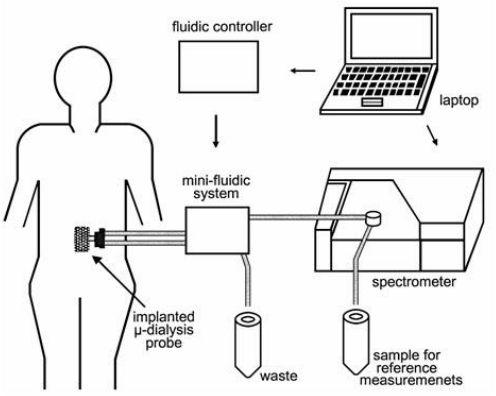

Fig. 1 Schematics of the advanced IR-spectrometric setup developed for continuous ex-vivo glucose monitoring in the dialysates from subcutaneously sampled interstitial fluid.

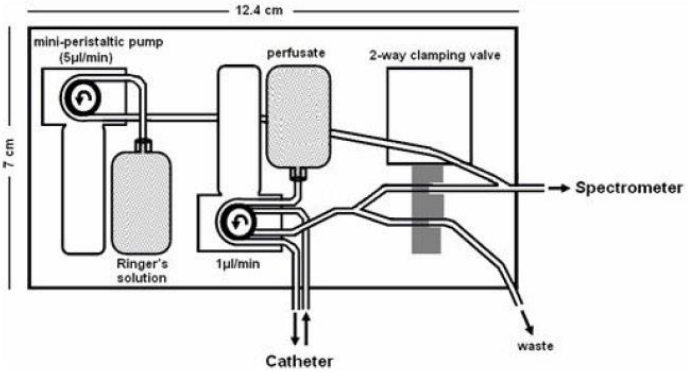

Fig. 2 Schematics of the mini-fluidic system developed for continuous ex-vivo glucose monitoring, consisting of two peristaltic mini-pumps and a magnetic two-way valve, for use with a subcutaneously implanted microdialysis catheter.

From the six minutes period with the sample flow through the cuvette (at a flow-rate of 1 µl/min), 12 spectra were recorded (accumulation over 30 s) and those with a threshold above 60 % of the maximum absorbance spectrum were sorted out for further processing. To reduce the noise level, a mean spectrum was calculated which is used to fit to the measured spectra using a least squares approach. The maximum value of the resulting scale factors was multiplied with the mean spectrum that provides a spectrum representing the plug concentration maximum with a reduced noise level compared with the individually measured ones. This spectrum was used for predicting the glucose concentration. As the spectrometer stability is high, further background spectra from two preceding measurements were also taken into account for spectral averaging.

This scheme allows successive measurements in 8 min intervals including spectral background and dialysate sample measurements. The advantage of this alternative fluidic set-up is that it is patient-wearable and the time lag between harvesting the dialysate and measurement has been reduced to 20 min, and in case of an increase of the microdialysis perfusion rate to 1.3 µl/min, this delay can be further cut down to 15 min. However, the spectral measurement time would also be reduced correspondingly to ~ 4 min. A further reduction would need significant miniaturisation of the mini-peristaltic pump and distribution tubing.

2.2 Instrumentation for glucose monitoring based on whole blood dialysates

The schematics of the continuous blood sampling including the extravascular microdialysis are presented in Figure 3. The complete continuous sensing device consists of two main components, i.e., an extravascular microdialysis system and the spectrometric sensor.

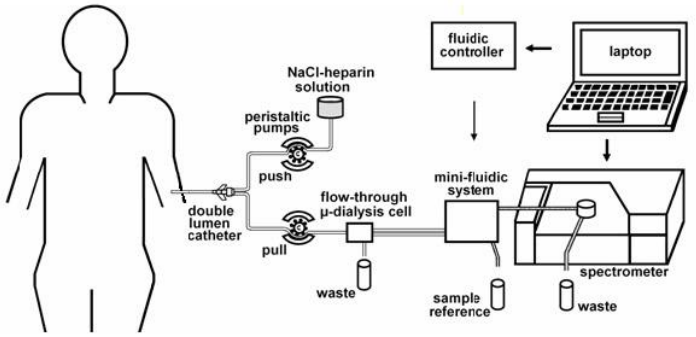

Fig. 3 Schematics of the IR-spectrometric setup developed for continuous ex-vivo glucose monitoring in the dialysates from whole blood.

Extravascular microdialysis system

The system consists of two peristaltic pumps (one in push and the other in pull-mode), a double lumen catheter (produced by Zier-Medizintechnik, Germany and distributed by MATEL Medizintechnik Ges.m.b.H. in Austria), and a flow-through microdialysis cell consisting of a planar dialysis membrane from GAMBRO Dialysatoren GmbH (Hechingen, Germany). The blood drawn from the double lumen catheter is mixed with a NaCl-Heparin solution (concentration: 100 ml NaCl 0.9 % and 1 ml Heparin 5000 IE/ml; 50 IU Heparin / ml solution; mixing proportion with blood 1:1) that is pumped by the peristaltic pump in a push-mode at a rate of 33 µl/min. The diluted whole blood is transported to the flow-through microdialysis cell by another peristaltic pump operating in a pull-mode at the flow rate of 66 µl/min. With the perfusate applied within the microdialysis cell, harvesting of compounds across the microdialysis membrane takes place at highest recovery rates, while the dialysate is transported to the sensor by an additional mini-fluidic system.

Sensor system

The sensor system consists again of an electronically controlled mini-fluidic system, the spectrometer including laptop-computer. The schematics of the mini-fluidic system are similar to that in Figure 2, apart from the fact that the peristaltic pump supplying the perfusate is external to the box. It consists of an electronically controlled mini-peristaltic pump operating in push mode at a flow rate of 5 µl/min and a 2-way clamping valve, a Ringer's solution containing bag, and an N-shaped Tygon tubing piece. The dialysate from the flow-through microdialysis cell is transported to the spectrometer by the N-tubing piece controlled by the 2-way clamping valve, similar to the system described above for the measurements in the subcutaneous interstitial fluid using the simplified fluidics. The clamping valve and the mini-peristaltic pump control the flow of the dialysate and the transport solution into the cuvette. When the clamping valve is switched to open-mode, the mini-peristaltic pump halts its function so that the dialysate is pumped into the cuvette. Once the spectra of the sample are measured, the clamping valve will be turned to the close-mode and the mini-peristaltic pump starts its operation again for cleansing the cuvette for the next cycle, beginning with a spectral background measurement. This scheme allows alternate measurements of 3.5 min for a background and 10.5 min for the dialysate sample. When the clamp valve is in closed mode, the dialysate flow is directed towards another vessel used for collecting the dialysate for reference glucose concentration measurements. In Fig. 4C, the timing of the glucose measurements is explicated.

As described above, the measurement cycle is divided into two parts. During the first part, seven background spectra are recorded of which the mean of the middle best four is used as the final background spectrum. Within the second part, 21 sample spectra were recorded. Three slices of 4 spectra measured for 30 s exist; each four spectra are averaged to provide a sensor reading so that three spectra are available for glucose concentration prediction. The three mean spectra are scaled due to a slight decrease in the glucose concentration at the beginning of the sample plug caused by the transport through fluidic dilution.

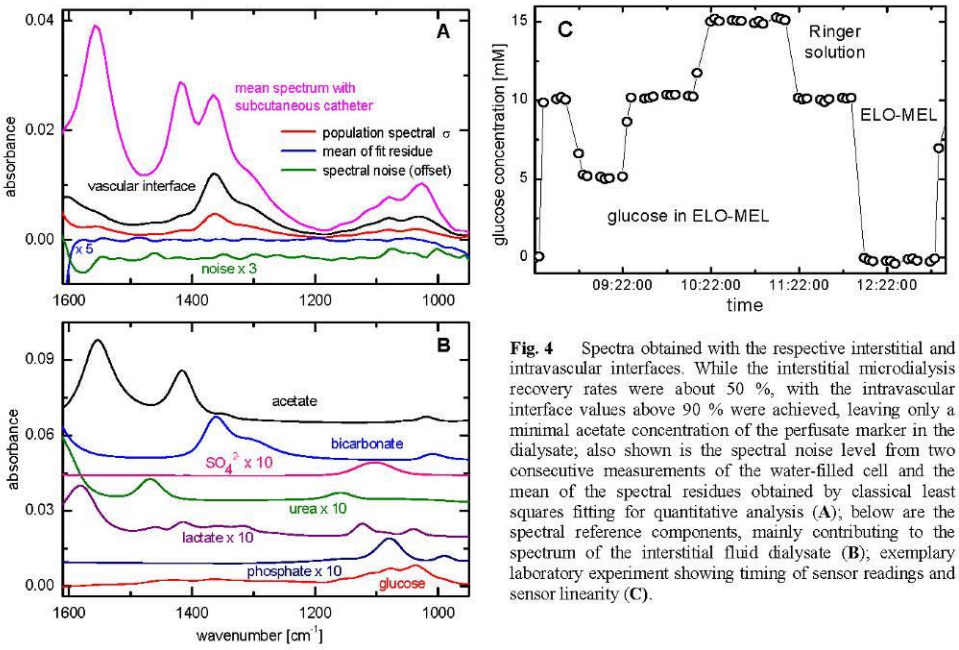

Fig. 4 Spectra obtained with the respective interstitial and intravascular interfaces. While the interstitial microdialysis recovery rates were about 50 %, with the intravascular interface values above 90 % were achieved, leaving only a minimal acetate concentration of the perfusate marker in the dialysate; also shown is the spectral noise level from two consecutive measurements of the water-filled cell and the mean of the spectral residues obtained by classical least squares fitting for quantitative analysis (**A**); below are the spectral reference components, mainly contributing to the spectrum of the interstitial fluid dialysate (**B**), exemplary laboratory experiment showing timing of sensor readings and sensor linearity (**C**).

2.3 Spectral recording and data evaluation

Infrared-spectra with a spectral resolution of 16 cm^{-1} were recorded using the IR200 mini-spectrometer. A measurement time of half a minute for accumulating the interferograms was chosen, and such spectra were further averaged for improving the signal-to-noise ratio. For interferogram apodisation, a Happ-Genzel function was applied. Technical details on the micro-cell can be found elsewhere.[13] The spectra of the dialysates were recorded at constant temperature maintained at 17°C and using a Ringer's solution for background measurements. Exemplary spectra obtained using the subcutaneous and intravascular interface, respectively, with the existing absorbance noise level are shown in Figure 4A. The reference spectra for Classical Least-Squares (CLS) that were used for modelling our experimental spectra are shown in Fig. 4B. These components are the major contributors to the dialysate spectra. Alternatively, we also employed Partial Least Squares (PLS) calibration modelling. Details on the Matlab-based in-house developed programs can be found in our previous paper.[15]

The determination of reference concentrations of dialysate samples, venous whole blood and heparinised diluted blood was carried out using a Hexokinase assay programmed on a Beckman Glucose Analyzer. The venous blood samples were collected under arterialized conditions with the arm resting in a hot box (50°C) and furnished with a venous port.

Ex-vivo experiments were carried out at the Center for Medical Research, Medical University Graz, Austria, on several type 1 diabetic individuals, with each experiment lasting 28 hours. In this paper, we report exemplary results from two patients using either a subcutaneous or a vascular body interface. The volunteers reached the Research Centre at 10:00 a.m., and the measurement procedure lasted from 12:00 noon until 5:00 p.m. of the next day. The volunteers did not start under fasting conditions and received their normal diet. For all subjects, a perfusate containing acetate with a concentration of 45 mM (ELO-MEL isoton, Fresenius Kabi, Graz, Austria) was used, enabling us for the simultaneous determination of the dialysis recovery rate, utilizing the fact that physiological concentrations of acetate found in human body fluids are much lower. The loss of this marker substance by diffusion into the interstitial tissue space or diluted blood can be used to quantify the transport processes across the microdialysis membrane. All measurement procedures had been approved by the local Ethics Committee of the Medical University Graz.

3. RESULTS AND DISCUSSION

3.1 Continuous subcutaneous interstitial glucose measurements

The body-interface, i.e. the subcutaneously implanted microdialysis probe, functioned in a similar way as reported in our previous papers.[15, 16] However, the fluidics transporting the dialysate to the spectrometer had been simplified and miniaturised (without the PC controlled switch valve including sample loop). Sensor readings were for every 10 minutes.

In Figure 5, the results from a PLS evaluation of the dialysate spectra aimed at optimising glucose and lactate measurements are presented. Besides the scatterplots, also a respective glucose and lactate spectrum are shown with the belonging optimal PLS regression vector for concentration prediction. There is a different number of PLS-factors needed for optimal concentration prediction of each compound – in part due to the broader spectral interval exploited for lactate, but the extreme values of the resulting regression vectors uniquely point to the specific spectral features of the glucose and lactate absorptivities. The relative scatter for the lactate metabolite concentration predictions is larger which is understandable from the much lower concentration regime as found for glucose.

A comparison of laboratory blood values and IR-sensor determined dialysate glucose concentrations using CLS calibration and taking further into account the microdialysis recovery rates is shown in Fig. 6A. The microdialysis recovery rates measured simultaneously are presented in Fig. 6B. The recovery rates are rather low, and for extreme cases we have observed values even from 15 to 85 %; as we can see in the figure, there are changes in the recovery rates that underline the necessity of an independent method for monitoring the dialysis efficacy. The relative difference of the sensor predicted values, scaled according using the recovery rate, to the reference blood glucose values were evaluated by Bland-Altman analysis (see Fig. 6C). The mean difference was only -0.5 % with a standard deviation of 8.3 %. The inset shows the histogram of the number of readings < 10 % error, ≥ 10 % and < 20 % error, and ≥ 20 % error. A fraction of 80 % of the sensor predicted values were within the < 10 % error interval proving the accuracy of the measuring device. Furthermore, the clinical applicability of the concentration readings were compared with the blood glucose

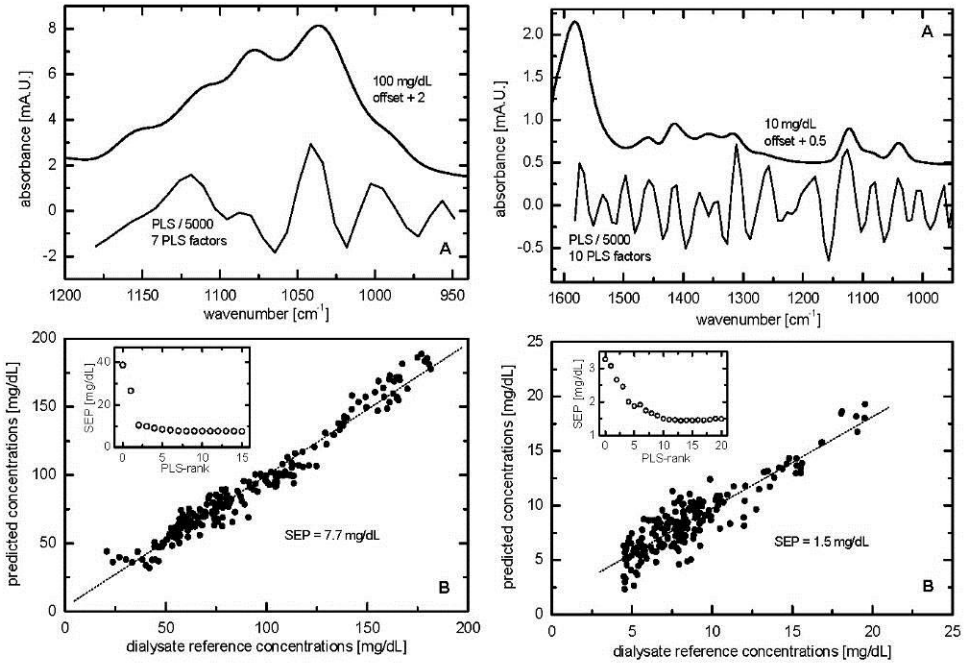

Fig. 5 Spectral absorptivities and PLS regression vectors for concentration prediction (**A**) and results from the PLS calibration showing a Standard Error of Prediction (SEP) of 7.7 mg/dL for glucose with 7 PLS factors using leave-one-out cross-validation (left side: **B**) and an SEP of 1.5 mg/dL for lactate with 10 PLS factors (right side: **B**) of the interstitial fluid dialysates measured using the subcutaneously implanted microdialysis probe in a type 1 diabetic subject (the insets in parts B show the SEP-dependency on the number of respective PLS-factors).

reference values using the Clarke Error Grid (CEG) analysis.[17] Values in zones A and B represent consistent or acceptable glucose concentration results. As illustrated in Figure 6D, the CEG analysis of the IR spectroscopically determined glucose concentrations listed 98.5 % values in the clinically accurate zone A and 1.5 % values in the acceptable zone B. In parts C and D an overview on the continuous sensor read-outs is provided by comparing the recovery corrected concentrations with interpolated whole blood concentration values by a spline function approximation. Another option is certainly to have the IR-sensor results compared with the much less frequent whole blood sample measurements around a fixed interval time window.

3.2 Continuous blood glucose measurements using dialysis of heparinised whole blood

A photograph of the instrumentation for the continuous blood glucose measurements using the dialysis of heparinised whole blood is shown in Fig. 7. The system is designed for a continuous blood sampling with further sample treatment using a planar flow-through microdialysis set-up including a 5 kDa custom made membrane. The microfluidics used here will limit the wastage of blood to 2 ml/h. The microdialysis unit is very efficient due to its counter current flow set-up. A positive aspect was that high dialysis recovery rates could be achieved, but for avoiding blood coagulation and reducing blood viscosity, a dilution to 50 % of the original whole blood composition had to be taken into account. The fluidic complexity is certainly a bit more error-prone compared to the simple one-peristaltic pump set up required for the subcutaneous micro-dialysis catheter – sensor coupling. Delay times were 8 min when investigating the sample transport

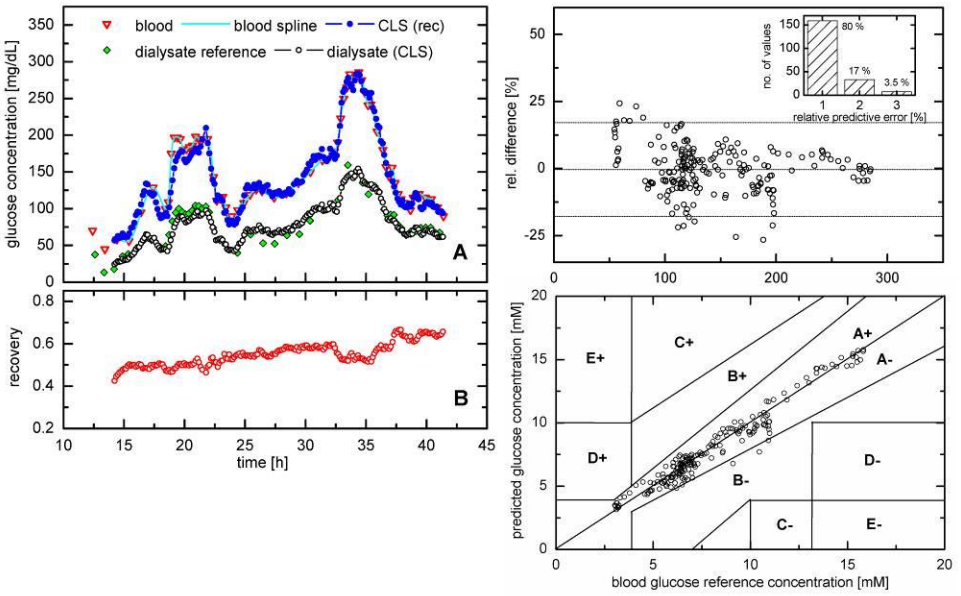

Fig. 6 Time course of continuous glucose monitoring with results from the IR-sensor with subcutaneous interstitial dialysate concentration read-outs and blood glucose values of a type 1 diabetic subject (**A**) including the microdialysis recovery rates (**B**); Bland-Altmann and histogram plots (inset) of the data from the reference plasma blood glucose and their corresponding interstitial dialysate MIR sensor concentration readings; inset: "1" on x-axis represents number of readings lower < 10 % error, "2" is number of readings within ≥ 10 % and < 20 % error, "3" is number of readings ≥ 20 % relative error **(C)**; CEG plot of the data from the reference plasma blood glucose concentrations and their corresponding interstitial dialysate MIR sensor concentration readings with 98.5 % values in zone-A and 1.5 % values in zone-B **(D)** (sensor features: calibration not required; 20 min lag time; 30 min running in period; simultaneous microdialysis recovery rate determination with the option of further components included such as lactate, urea, acetate, bicarbonate and others).

and spectrometer measuring times, which can be tolerated within a closed loop approach in combination with an insulin pump.

An exemplary temporal glucose profile measured in one type 1 diabetic subject is presented in Figure 8. The performance of the sensor evaluated using Bland-Altman, histogram and CEG plots is also presented. The mean relative difference was 8.6 % with a standard deviation of 11.9 %. In total 48 % of the sensor predicted values were within < 10 % error, 34 % were within the ≥ 10 % and < 20 % error interval and 18 % were above ≥ 20 % error. The CEG analysis showed that 92.5 % of the values were in the clinically accurate zone A and 7.5 % values in the acceptable zone B. The systematic deviations observed between whole blood measurements and sensor readings must be discussed. The good agreement between the diluted blood and recovery corrected dialysate glucose values suggests deviations from assay linearity for the Beckman Analyzer for high glucose concentrations above 250 mg/dL as measured in plasma from whole undiluted blood.

A positive feature of the system with the intravascular body interface is the low complexity of the involved microdialysis device using a simple planar semi-permeable dialysis membrane, compared to the costly CMA 60 catheter as used for subcutaneous adipose tissue implantation. This also allows the use of sterilizable and reusable parts, although integrated disposable components will be favoured for use within the intensive care environment. Further results on the multi-component assay capability of the MIR-sensor system will be reported in a future paper.

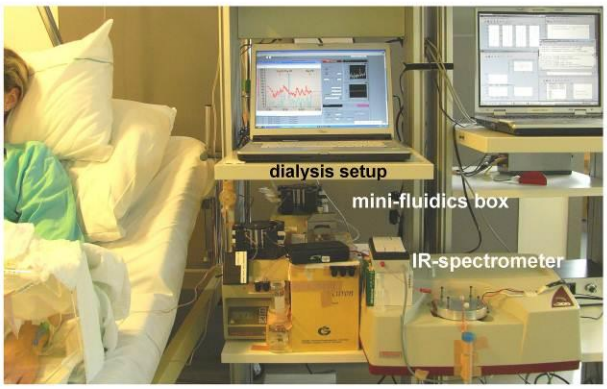

Fig. 7 Photograph of the hardware of the IR-spectrometric setup developed for the continuous ex-vivo glucose monitoring in the dialysates from whole blood. Similar setup was also applied for the continuous subcutaneous glucose monitoring in the subcutaneous interstitial fluid but replacing the external dialysis setup by a CMA 60-catheter and a patient wearable mini-fluidic system as shown in Figure 2.

We have monitored also the bicarbonate concentrations in the dialysate, which can give a good indicator for the simultaneous determination of the blood dilution rate by infrared spectrometry. Another option for improving the total system reliability is certainly an improvement of the robustness of the blood sampling branch. Further parameters of interest for monitoring the physiological status of a patient would be pCO_2 and pH measurements, which have been provided in the past by separate blood gas measurements. Finally, it can be reported that the MIR-sensor readings have been successfully used within semi-closed loop experiments for controlling the insulin infusions required for glycemic control in type 1 diabetic volunteers. For safety reasons, the 15 min interval average sensor readings have been compared with venous whole blood measurements based on the Beckman Analyzer, and a manual transfer of the actual blood glucose concentration value as input for a laptop-computer based software as decision supporting system was used.

4. CONCLUSIONS

A high correlation between sensor estimated glucose concentrations based on microdialysis versus arterialized venous blood glucose levels can be achieved using our technology, which allows an accurate simultaneous determination of the microdialysis recovery rates that are varying over time, which were even observed for the microdialysis device equipped with a planar dialysis membrane – although at a much smaller rate. This assay feature has been implemented within the infrared spectrometric approach developed within the CLINICIP project. The variable recovery rates experienced with subcutaneously implanted catheters are one of the reasons, apart from inherent sensor drift observed for electrochemical sensors, that frequent recalibrations are required if most reliable blood glucose monitoring is envisaged. On the other hand, a direct measurement of blood glucose as realized using a vascular body interface is closer to the holy grail of the medical community with regard to realize glycemic control in critically ill patients than using the adipose tissue implanted subcutaneous micro-dialysis catheter in combination with an ex-vivo sensor arrangement.

ACKNOWLEDGEMENTS

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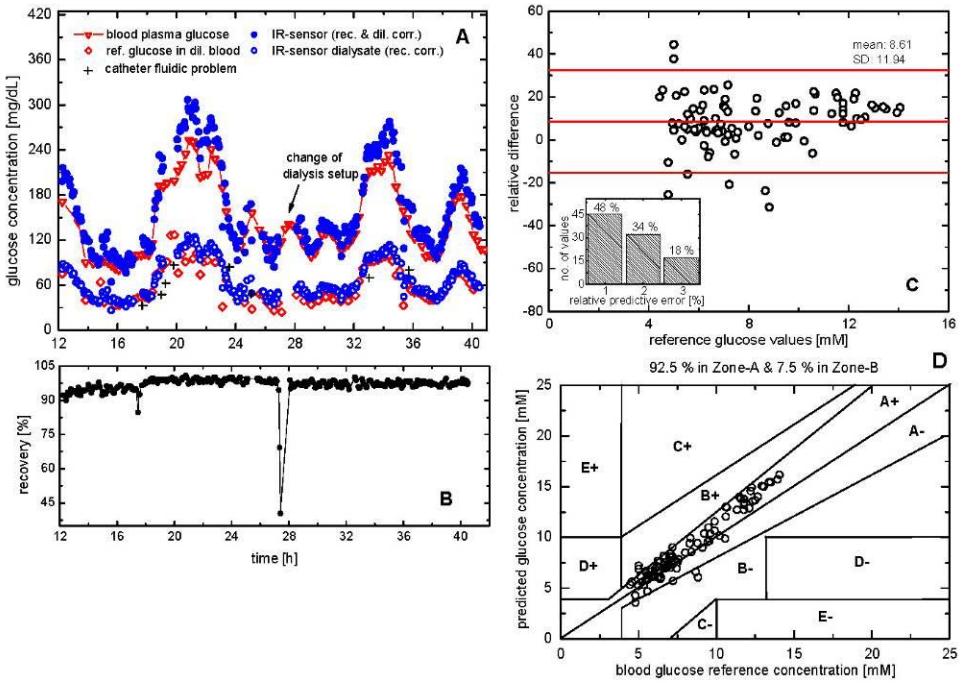

Fig. 8 Time course of continuous glucose monitoring with results from the IR-sensor with diluted whole blood dialysate concentration read-outs and blood glucose values of a type 1 diabetic subject (**A**) including the microdialysis recovery rates (**B**); Bland-Altmann and histogram plots (see inset) of the data from the reference plasma blood glucose and their corresponding interstitial dialysate MIR sensor concentration readings **(C)**; inset: "1" on x-axis represents number of readings below < 10 % error, "2" is number of readings within ≥ 10 % and < 20 % error interval, "3" is number of readings with a ≥ 20 % relative error; CEG plot of the data from the reference plasma blood glucose concentrations and their corresponding dialysate MIR sensor concentration readings with 92.5 % values in Zone-A and 7.5 % values in Zone-B **(D)** (sensor features: calibration not required; 8 min lag time; 30 min running in period; for other features, see caption of Figure 6).

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Continuous blood glucose monitoring by infrared spectrometry as an important tool in clinical research and therapy for improving glycaemic control in diabetic and critically ill patients

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Abstract

For clinical research and therapy, continuous blood glucose monitoring is an important tool for improving glycaemic control in diabetic and critically ill patients. A mid-infrared spectrometer in combination with a body interface for continuous glucose monitoring has been developed, delivering the reliability as needed for intensive care. The developed prototype has been tested successfully for online monitoring of glucose and other metabolites in healthy and type 1 diabetic subjects. Monitoring of subcutaneous interstitial fluid or whole blood dialysate up to 28 h was realized with concentration readings at 5 or 10 min intervals. The body interface between subject and glucose sensing device was either an implantable μ-dialysis catheter or an extra-corporeal whole blood μ-dialysis device. The spectrometric glucose monitoring technology provides long-term reliable results without the needs of sensor recalibration, which can be used as input data for an artificial pancreas system using a programmable insulin pump in combination with an appropriate algorithm for its control.

Keywords: clinical chemistry, continuous glucose monitoring, infrared spectroscopy, micro-dialysis

Introduction

Diabetes mellitus is widely acknowledged as a growing health problem. Type I develops when the pancreatic beta cells are destroyed by the body's immune system, and Type II can be manifested when the tissue cells become insulin resistant. The major complications of diabetes mellitus include ketoacidosis, diseases affecting microcirculation (e.g., nephropathy, neuropathy, and retinopathy) and macrocirculation (e.g., atherosclerosis, coronary heart disease, stroke, and peripheral vascular disease) and even death [1]. The benefits of tight glycaemic control in diabetic patients have been well

documented since the completion of the Diabetes Control and Complications Trial (DCCT) studies [2-4]. Studies indicated that intensive insulin therapy in diabetic patients can dramatically delay the onset of serious complications. Most diabetic patients are using blood glucose self-monitoring (SMBG) devices for episodic surveillance of their glucose levels and adjustment of their insulin dosage to achieve normoglycaemia. Similar needs as found essential for diabetic patients have been requested for patients under intensive care [5, 6].

Hyperglycaemia over extended periods has been diagnosed in patients faced with critically ill conditions such as trauma, haemorrhage, burns, hypoxia, infections, sepsis and shock. The stress of critical illness induces glucose counter regulatory hormones and a number of alterations in the carbohydrate metabolism, resulting in peripheral glucose demands, enhanced hepatic glucose production, insulin resistance, and relative insulin deficiency [5]. In addition, several commonly employed clinical interventions, such as corticosteroids, vasopressors and enteral (or parenteral) nutrition, further predispose these patients to elevated blood glucose levels. In a variety of clinical settings, stress hyperglycaemia has been associated with adverse clinical outcomes such as myocardial infarctions, polyneuropathy and multi-organ failure. Studies indicate that strict glycaemic control (lowering the blood glucose levels to 80-110 mg/dl) reduces intensive care unit mortality, morbidity and duration of hospital stay. According to the needs for intensive care patients, different monitoring devices for blood sugar measurement have been developed, mainly based on electrochemical enzymatic biosensors.

Over the past few years, there has been enormous progress in research to find improved instrumentation for glycaemic control. These include device miniaturisation, sensor fabrication, disposable sensors, lancing techniques, reduced sample volume (down from several microlitres to nanolitres), and minimisation of interferences from chemical, atmospheric and environmental factors. Other aspects are concerned with alternate or multiple-site testing, measuring one or more components, automatic calibration, test memory, computer connectivity, and diabetes management software, but clinical accuracy and performance are top of the list of improvements achieved. When undergoing intensive insulin therapy, current monitoring requires blood sampling, e.g. by finger pricking, several times a day [1, 6]. The limited information provided by these episodic glucose monitors makes it difficult to accurately adjust the treatment. Several invasive and minimally invasive glucose technologies (see Ref. [1] for more information) have also been developed for continuous glucose sensing but not yet established for optimal glycaemic control in diabetic and critically ill patients [1, 8]. The main disadvantage of these technologies is that the temporal glycaemic profiles obtained from these devices are not reliable unless they are used repeatedly in combination with traditional SMBG devices for recalibration.

Non-invasive spectroscopic methods have also been experimented by several research groups for blood glucose sensing. The optical spectroscopic methods reported were vibrational spectroscopy (including near-infrared, mid-infrared and Raman), fluorescence spectroscopy, thermal emission

spectroscopy, optical coherence tomography, polarimetry, and techniques based on the thermal properties of tissues [1]. While many non-invasive approaches have shown initial promise in the past, they are limited due to high background absorption of water, baseline shifts, instrumental drifts, lack of sensitivity, overfitting, poor precision, and the effect of blood flow and its pulsatile nature on the measurements.

For the benefit of intensive care patients, the recently developed options for continuous glucose monitoring are sensor systems based on continuous sampling via a venous port or a subcutaneously implanted microdialysis probe with ex vivo detection. Infrared spectroscopy in combination with the available microfluidic technology is a promising method for continuous glucose monitoring. A fluidic system can be used to load the sample, e.g. from a body interface, into a flow-through cell, which can be arranged within the sample compartment of a conventional spectrometer.

Infrared spectroscopy has already been successfully used for the study of various biomedical tissue or biofluid samples [9]. The analysis takes advantage of the fact that a multitude of analytes can be quantified simultaneously and rapidly without the need for reagents using the absorption of electromagnetic radiation with the biosample. Recent trends show that dry film measurements by infrared spectroscopy could revolutionize the analytical assays in the clinical chemistry laboratory. Results on minimal-invasive glucose monitoring in diabetics even with nanoliter sample volumes have been presented in the past [10].

Another hot topic is the development of glucose monitoring technology for diabetic and critically ill patients as part of an artificial pancreas system. Infrared spectroscopy with a micro-cell of sub-microliter internal volume can be used for drift-free patient monitoring. Results from continuous measurements with whole blood show the needs for improving the biocompatibility of cell window materials to avoid the adsorption of cellular blood components for long-term usage. On the other hand - without any complications arising from the missing bio-compatibility of the cell materials for blood, transmission spectroscopy can be reliably applied for patient monitoring using biofluid harvesting by means of a subcutaneously implanted micro-dialysis catheter or by dialysis of continuously sampled whole blood [11, 12].

Using this approach, the biofluid matrix can be significantly simplified, since large molecular mass components such as proteins can be separated from the sample to be analyzed. The method also allows the determination of metabolites such as urea, lactate and others based on multivariate calibrations. Another advantage is the simultaneous assessment of the variable dialysis recovery rate by using a marker substance in the perfusate for monitoring the losses of this compound through the dialysis process [12]. Using the dialysate concentrations and the previously established relationship between dialysis marker losses and glucose recovery, an accurate quantification of subcutaneous interstitial or whole blood concentrations is made possible, which will be further illustrated.

Experimental

The schematic set-up for glucose monitoring in subcutaneous interstitial fluid dialysates is shown in Figure 1A. It requires an implantable microdialysis catheter CMA 60 from CMA Microdialysis AB (Solna, Sweden), a custom-made patient wearable mini-fluidic system that consists of two mini-peristaltic pumps (one for transporting perfusate to the catheter at 1 µl/min, and another for advancing Ringer's solution at 5 µl/min for sample transport to the spectrometer and back ground measurements) and a two-way magnetic switch valve (Takasago Electric, Inc., Nagoya, Japan), ancillary electronics for controlling the fluid transport, a laptop computer and an IR200 mini-spectrometer (Thermo Electron Corporation, Madison, U.S.A) equipped with a room-temperature thermal DTGS detector and a Peltier-cooled transmission micro-cell with CaF_2 windows and a polyethylene spacer providing a 30 µm optical sample pathlength. The internal cell volume was 0.6 µl. Polyetheretherketone (PEEK) tubings with respective outer and inner diameters of 800 µm and 200 µm were connected to the in- and outlet of the cell using special PDMS-seals for avoiding dead volume within the cuvette.

Spectroscopic air bubble detection by monitoring the opaque spectral intervals that were due to strong water absorption was implemented, and subsequent air bubble removal from the flow-through micro-cell by using software for activating the fluidic system for cell flushing was also active to enable for reliable photometry. However, this safety feature was never invoked after we changed from slightly elevating the cell temperature by heating to Peltier-cooling to 18°C. The schematics of the continuous blood sampling including the extravascular microdialysis unit are presented in Figure 1B. For this set-up, the complete continuous sensing device consists of two main computer-controlled components, i.e., a blood sampling device in combination with an extravascular microdialysis system and the spectrometric sensor [11].

A measurement cycle of the automatic measurement system started with reference measurements of the transmission micro-cell filled with Ringer's solution followed by consecutive dialysate measurements. By calculating absorbance spectra from sample and reference spectra recorded shortly after another, the quality of spectra is high, since spectrometer drift or changes in atmospheric absorptions within the spectrometer can be minimized, allowing also successive dialysate measurements in 10 min intervals. The scheme for the continuous monitoring of dialysates from heparinized whole blood consisted of alternate measurements of 3.5 min for a spectral background using the micro-cell rinsed with Ringer's solution and of 10.5 min for the dialysate samples providing glucose concentration readings for every five minutes on average.

Details on the Matlab-based in-house developed programs for quantitative multivariate analysis of the dialysate absorbance spectra can be found in our previous publication [12]. In particular, the spectral features within the so-called fingerprint interval of the mid-infrared spectral range have been exploited for quantitative determination. Some reference spectra for the Classical Least-Squares (CLS) fitting that were used for the linear modelling of our experimental spectra are

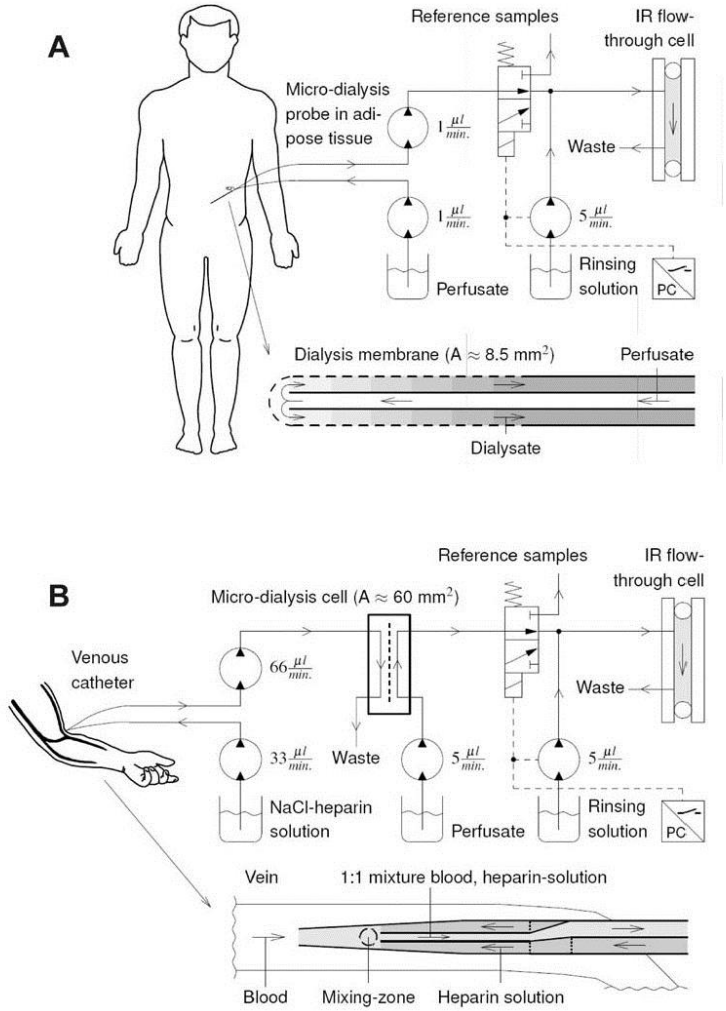

Fig. 1: **A** Harvesting of dialysates from subcutaneous interstitial fluid and transport to the micro-cell; **B** Continuous blood sampling, microdialysis of diluted blood and dialysate transport to the micro-cell.

shown in Fig. 2. A non-linear baseline and an atmosphere spectrum for compensating slight changes in water vapour absorption within the spectrometer between sample and reference measurements had also been taken into account for spectral fitting. Spectral resolution for recording dialysate and background spectra, as well as reference spectra of the dialysate components was 16 cm^{-1}.

5

Ex-vivo experiments, each lasting 28 h, were carried out at the Center for Medical Research, Medical University Graz, Austria, on healthy and type 1 diabetic individuals. The volunteers arrived at the Research Centre in the morning, and measurements lasted from 11:00 noon until usually 5:00 p.m. of the next day. The volunteers received four regular meals. For all subjects, a perfusate containing acetate with a concentration of 45 mM (ELO-MEL isoton, Fresenius Kabi, Graz, Austria) was used; this enabled us for the simultaneous determination of the dialysis recovery rate, since the loss of this marker by diffusion into the interstitial tissue space or diluted blood was used to quantify recovery rates from the microdialysis process [12]. All measurement procedures had been approved by the local Ethics Committee of the Medical University Graz.

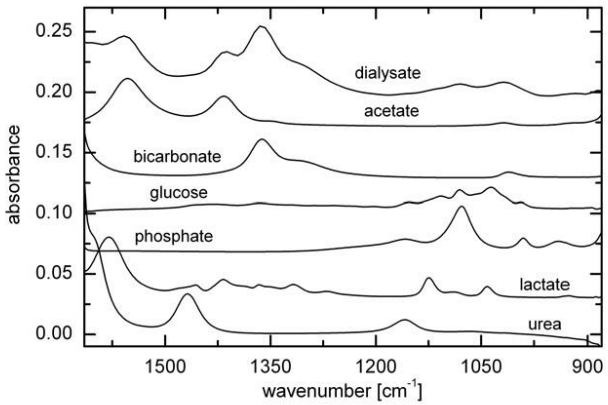

Fig. 2: Dialysate, recovery marker (acetate) and substrate spectra used for quantitative analysis by classical least squares fitting (measurements by transmission, internal cell volume 0.6 µl, optical pathlength 30 µm).

Results and Discussion

The analytical performance of the bed-side system developed here relies very much on the simultaneous determination of the dialysis recovery rate, which is not possible using conventional biosensor technology currently applied for glucose monitoring. By this approach, a high correlation of values determined by infrared-spectrometry to reference measurements based on venous blood has been achieved. The multi-assay capability can be illustrated by the examples of a dialysate spectrum, the acetate marker substance and important substrate spectra that have been used for quantitative analysis by CLS-fitting of dialysate component spectra (see Fig. 2).

Prior to the experiments on human subjects for assessing the sensor system performance, a protocol was established for testing sensor linearity, accuracy, precision, and characterising drift effects over extended operation times of at least 72 h [13]. The sensor linearity was tested using aqueous

solutions at different concentrations between 2.5 and 22 mM, prepared gravimetrically in Ringer's solution. The correlation coefficient was R=0.9998. For an experiment with serum ultrafiltrates of different glucose concentrations between 5.2 and 15.8 mM, lasting for more than 90 h, the relative bias to the reference values varied between 2.0 and 3.8 %, while the coefficient of variation was found between 2.1 and 6.0 %. Sensor drift over such a period of time was less than 1 %. These results suggest the reliability of the device for its continuous application in the clinical environment.

In Fig. 3A, the temporal glucose concentration profiles in venous whole blood and the glucose concentration values as obtained by the subcutaneously implanted micro-dialysis catheter and IR-spectrometry, using the simultaneously derived dialysis recovery rate (see lowest trace), is illustrated. The recovery rates are rather low, and for extreme cases we have observed values even from 15 to 80 %; as we can see in the figure, there are changes in the recovery rates that underline the necessity of an

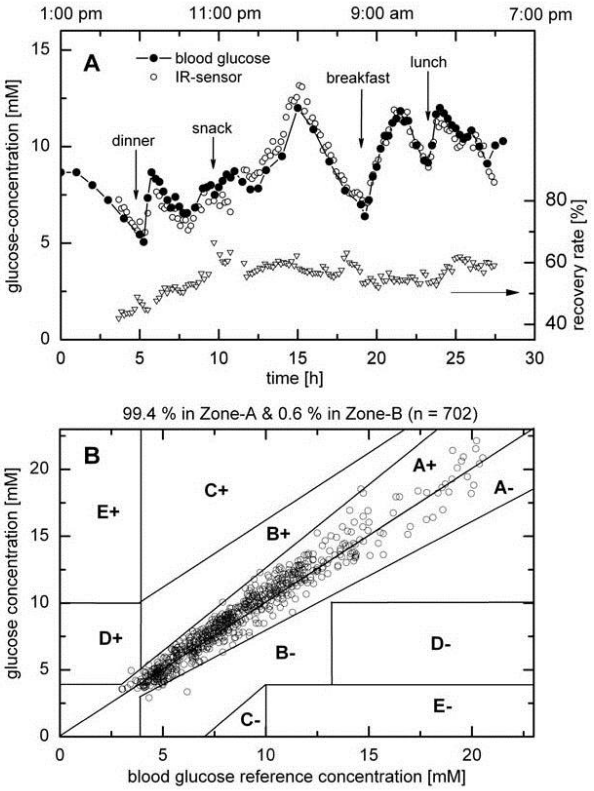

Fig. 3: **A** Time course of glucose monitoring of a diabetic subject with results from the IR-sensor with recovery-corrected subcutaneous interstitial concentration read-outs and blood glucose values including dialysis recovery rates; **B** Clarke Error Grid plot of sensor predicted glucose and reference blood glucose concentration values from a healthy and five diabetic volunteers.

independent method for monitoring the dialysis efficacy. The results for a healthy and five diabetic volunteers, which were achieved with the subcutaneous micro-dialysis body interface, have been summarized in Fig. 3B. For testing the clinical applicability, the concentration readings were compared with the blood glucose reference values using the Clarke Error Grid (CEG) analysis. Values in zones A and B represent consistent or acceptable glucose concentration results [14, 15]. As illustrated in Fig. 3B, the CEG analysis of the glucose concentrations determined by IR-spectrometry listed 99.4 % values in clinically accurate zone A and 0.6 % values in the acceptable zone B.

An exemplary glucose concentration profile of a diabetic subject monitored by extra-corporeal whole blood dialysis is shown in Fig. 4A, whereas the summary of results for five diabetic subjects is displayed in Fig. 4B. The CEG analysis showed that 97.2 % of the values were in the clinically accurate zone A and 2.8 % values in the acceptable zone B. A positive feature of the system with the

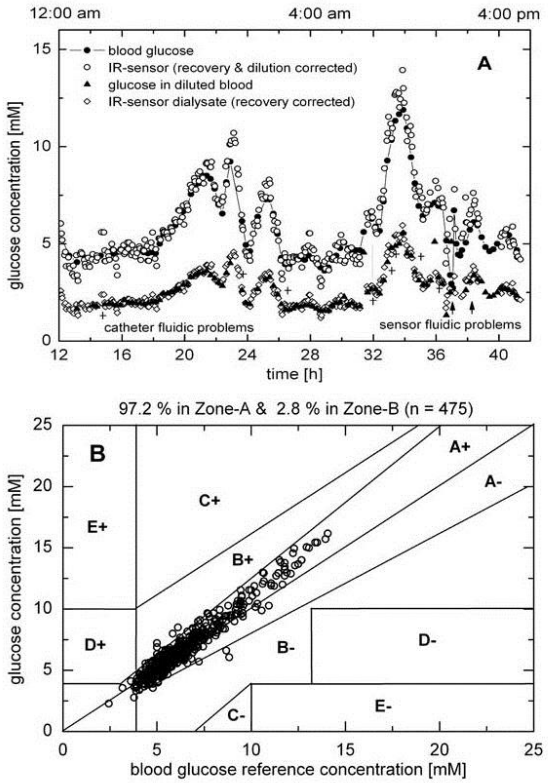

Fig. 4: **A** Exemplary temporal glucose concentration profiles of a diabetic subject monitored by extra-corporeal whole blood dialysis (see also text); **B** Clarke Error Grid plot of sensor predicted glucose and reference blood glucose concentration values from five type 1 diabetic volunteers.

intravascular body interface is the low complexity of the involved microdialysis device using a simple planar semi-permeable dialysis membrane, compared to the expensive CMA60 catheter as used for subcutaneous adipose tissue implantation. This allows also the use of sterilizable and reusable parts, although integrated disposable components will be favoured for use within the intensive care environment.

As a result of our research, an automated, long-term reliable bed-side spectrometric device has been developed for continuous monitoring of blood glucose with recommendable application to intensive care patients, replacing possibly less efficient electrochemical biosensors designed for single-component monitoring. The device performance has been tested in many clinical measurement campaigns.

Prospects for spectrometer miniaturization are promising and will enable even for wearable devices applicable for diabetic patient self-monitoring of blood glucose. The applicability of the developed sensor towards glycaemic closed loop control had also been tested. The sensor readouts were used as input for manually controlling the patient glucose levels by appropriate insulin dosage advised by the eMPC algorithm developed by R. Hovorka and his group from Cambridge University [7, 16], and good performance was achieved. For a recent review on dynamics of insulin concentration in the blood and modelling, see also [17].

Furthermore, the computerised and automated sensor system has been explored for its multi-component capability for glucose, urea, lactate, acetate, bicarbonate, pCO_2 and pH of the interstitial biofluid with focus also on the physiological buffer system or for drug monitoring including pharmaco-kinetic studies. The applicability of the developed system for on-line monitoring of urea in patients undergoing dialysis treatment has also been successfully tested by in-vitro experiments [18]. There are several potential benefits of such a sensor. In addition to the dialysis adequacy, problems affecting dialysis efficiency such as poor dialyser performance can also be identified and corrected. Besides the clinical use, applications in other areas such as bioreactor monitoring (fermentations and cell-cultures) can also be envisaged.

Conclusions

The EU funded project CLINICIP (Closed Loop Insulin Infusion for Critically Ill Patients; http://clinicip.org) aimed to develop a low–risk monitoring and control system allowing metabolic control in intensive care units. In addition to the approach of measuring glucose concentrations in the blood, a minimally invasive technique to assess glucose concentrations in the subcutaneous tissue was evaluated. In conclusion, it has been shown that bed-side instrumentation using a small Fourier-transform infrared spectrometer is applicable for point-of-care diagnostics in the hospital, focussing on blood glucose measurements, but which is certainly able to provide also information on other metabolites and blood gas such as carbon dioxide. Based on continuous measurements of our sensor

systems, an adaptive control algorithm generated advice, representing thus a decision support system. Within a closed loop system, intensified insulin treatment has utilized those calculations leading to a regulation of blood glucose in type 1 diabetic patients. Eventually, the system provides the missing link of a reliable biosensor within an artificial pancreas system for critically ill patients.

Acknowledgements

The collaboration within the European project consortium and the financial support by the European Commission (CLINICIP project, contract no. 506965, 6th Framework Programme) is gratefully acknowledged. Financial support by the Ministerium für Innovation, Wissenschaft und Forschung des Landes Nordrhein-Westfalen and the Bundesministerium für Bildung und Forschung is gratefully acknowledged by the authors from ISAS.

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CHAPTER VII

“Closing the loop” – Integration of a model predictive control algorithm to form a semi-automatic closed-loop blood glucose control device and its evaluation in a clinical study

This chapter is mostly taken from a previously published article (Franz Feichtner et al. 2011). It is complemented by so far unpublished data.

1. Introduction

The previous chapters described the development and the technical and clinical evaluation of a vascular based body interface, which continuously extracts a protein-free dialysate from whole blood using an extravascular planar flow-through microdialyser (PFTMD). Glucose concentration in the dialysate was measured using online glucose sensors that were also developed and evaluated in healthy and type-1 diabetic subjects in the course of the CLINICIP project (H. M. Heise, Damm, Manfred Bodenlenz, et al. 2007b; H. M. Heise et al. 2008; R Schaller et al. 2009; Pasic et al. 2006; Pasic et al. 2007). In a clinical study the amperometric and spectrometric glucose sensor were connected to the dialysate outflow of the vascular body interface to form a continuous glucose monitoring system. In this study the spectrometric glucose sensor outperformed the amperometric glucose sensor technically and clinically and was thus used in the next study. Here – following a stepwise approach – the continuous glucose monitoring system was combined with the last component that is necessary to close the glycaemic control loop: the control algorithm, which is based on the model predictive control (MPC) paradigm and generates advice with respect to insulin infusion rates. It was originally developed and tested in subjects with T1D (Roman Hovorka, L. J. Chassin, et al. 2004) and was later used in the critically ill (Johannes Plank, Blaha, Cordingley Jeremy, et al. 2006; Kulnik et al. 2008).

In this chapter a feasibility study is presented where these components were combined for the first time to form a closed-loop device. Before approaching the target population, this feasibility trial was performed in T1DM volunteers, evaluating safety and performance issues that apply to ICU patients while trying to establish normo-glycaemia semi-automatically.

2. Material and methods[7]

2.1 Study protocol

The 30-hour feasibility studies were performed in four type 1 diabetes mellitus volunteers (body mass index: 25.8 ± 6.3 kg/m²; age: 31 ± 8 years; three male; diabetes history: 11.4 ± 9.0 years; hemoglobin A1c: 7.2 ± 0.8%) in a supine position. Each subject received four standardised meals (dinner: 6 p.m., snack: 10 p.m., breakfast: 8 a.m., lunch: 12 p.m.) sized 37, 29, 36, and 31 g carbohydrates (CHO), respectively.

[7] Partly taken and adapted from (Franz Feichtner et al. 2011)

As described in the previous studies, peripheral venous blood was continuously withdrawn from a standard intravenous line at 2 ml/hour and pumped to the extracorporeal membrane-based microdialyser by which a protein-free matrix was generated. Dialysate was analysed for glucose concentrations using the online infrared spectrometric glucose sensor, which was located directly at the dialysate-outflow of the PFTMD device and calculated an averaged glucose value each five minutes. Each 15 minutes these glucose sensor concentrations were entered into an algorithm together with the carbohydrate-content of each meal. For safety reasons, only online sensor values within ±20% of the reference plasma glucose concentrations were used as input for the algorithm. Otherwise, venous plasma glucose concentrations measured with a Beckman glucose analyser were used.

In this study hypoglycaemia was defined as blood glucose levels below 50 mg/dl, such as suggested in the Leuven studies (Greet van den Berghe et al. 2001).

The study received approval from the Ethics Committee of the Medical University of Graz. A picture of the bedside study set up can be seen in **Figure 29**.

2.2 Model predictive control (MPC) algorithm

In the present study, a control algorithm based on a MPC paradigm (Bequette 2005) was used. The algorithm is based on a model of glucose regulation in type 1 diabetes mellitus (T1DM) patients described in detail by Hovorka and colleagues (R Hovorka et al. 2004). The MPC controller was originally developed and tested in subjects with T1DM (Roman Hovorka, L. J. Chassin, et al. 2004) and a modified version was used in the critically ill (Johannes Plank, Blaha, Cordingley Jeremy, et al. 2006; Kulnik et al. 2008). The present study uses the updated controller in a population for which it was originally developed. According to the Leuven insulin titration guideline (Greet van den Berghe et al. 2001), the algorithm was initialised to 80 and 110 mg/dl as lower and upper limits of normo-glycaemia. The suggested insulin dosage was then administered by a standard IV-insulin infusion pump.

2.3 Data analysis

Glucose monitoring system

In this study the glucose monitoring system was evaluated as a whole, i.e. the combined system of PFTMD and infrared spectrometric glucose sensor. As in the previous chapters, the technical evaluation parameters include the Pearson's coefficient of correlation (R), mean difference

(MD), mean absolute difference (MAD), mean relative difference (MRD), mean absolute relative difference (MARD), %PRESS and ISO-criteria (ISO MET?) as suggested by Wentholt et al. (Wentholt et al. 2008). Evaluation parameters are given as means ± standard deviation for each subject and for all subjects in summary.

The clinical performance of the glucose monitoring system was evaluated in an insulin titration error grid analysis (Martin Ellmerer et al. 2006) using paired glucose readings of the prospectively one-point calibrated sensor and reference blood samples. Data analysis was performed using Microsoft Excel (Microsoft, Inc., Redmond, WA, USA).

Glycaemic control evaluation

Glycaemic control was evaluated using the following parameters: time to target, time in target, min-, max-, mean-, daytime-, nighttime-BG, peak post-prandial glucose concentrations, pre-meal mean BG concentration and the hyperglycaemic index [HGI, (Vogelzang et al. 2004)], which is an appropriate measure of glycaemic control that was especially developed to assess glycaemic control in ICU patents. The HGI can be calculated as the area under the curve above the upper limit of normal (here: 110mg/dl) divided by the total study duration. It can also be applied to quasi-continuous data.

Clinical analysis

To assess the closed loop system the Chassin grading system was applied (L. J. Chassin et al. 2005): this grading system is used to assess closed-loop systems clinically and was presented by Chassin and colleagues. Based upon the absolute plasma glucose concentration, this grading system differentiates outside meal and postprandial glucose conditions using 6 grades (A-F) (L. J. Chassin et al. 2005).[8]

[8] excellent (A) and good (B) glucose control: no need for a corrective therapeutic action; suboptimal control (C) with recommending a corrective action; poor control (D) requiring a corrective action; very poor (E) and life-threatening (F) control, requiring immediate corrective action or external assistance.

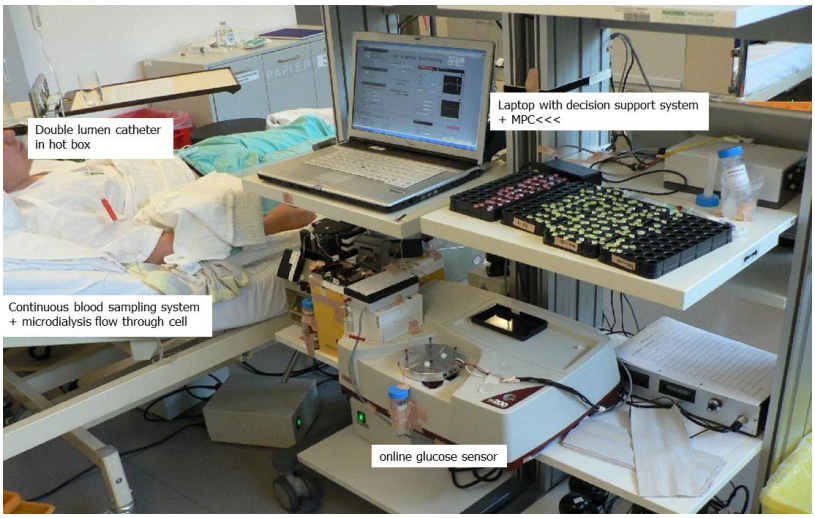

Figure 29: Photograph of the bedside study set up.

3. Results[9]

Reference blood glucose concentration and corresponding insulin infusion time profiles of the four investigated T1DM subjects (A–D) are illustrated in **Figure 30**.

3.1 Glucose monitoring system

Reference glucose concentration values are printed in **Figure 31** together with online concentration values of the infrared spectroscopic glucose sensor. Online glucose sensor values are shown as they were read out during the study. It can be seen that the online glucose sensor nicely follows the reference glucose concentration, independent of the dynamic range and the trial duration.

The evaluation results of the glucose monitoring system are presented in **Table 6**.

The overall coefficient of correlation was found to be 89.0 ± 0.04. MD, MAD, MRD and MARD averaged -2.3 ± 14.5 mg/dl, 9.8 ± 10.9 mg/dl, -2.2 ± 13.8 mg/dl and 9.2 ± 13.8 mg/dl, respectively. %Press was found to be 12.6 ± 2.9 % in average. ISO criterion was met in 381 of 420 total paired glucose values (REF vs. online glucose sensor value), which is equivalent to 90.7%.

Deriving valid glucose concentrations from the combined body interface online-sensor system failed 24 times (5.4%). Valid online glucose concentrations exceeded the critical 20% deviation

[9] Partly taken and adapted from (Franz Feichtner et al. 2011) and complemented by so far unpublished data

threshold in an average of 8.8% (min: 1%; max: 17%). In these cases the online glucose sensor value was overruled and the reference glucose concentration was entered into the algorithm.

The clinical performance evaluation of the combined glucose monitoring system (body interface + spectroscopic online sensor) using the insulin titration error grid analysis finds 98.8% of all glucose data pairs in the Acceptable Treatment (AT) area and 5 of 420 data pairs (i.e. 1.2%) in the Unacceptable Violation area, compare **Figure 32**.

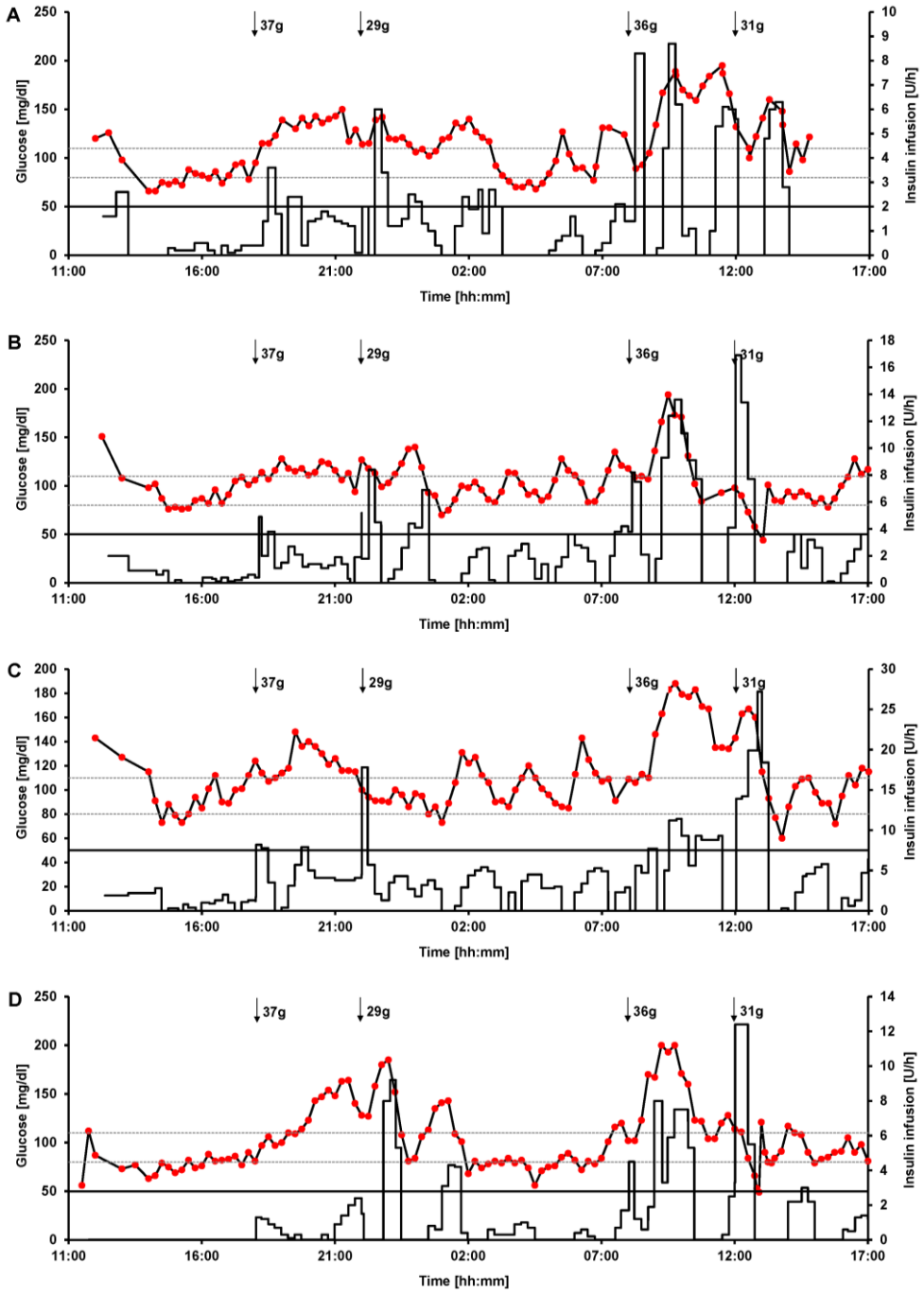

Figure 30: Reference glucose concentration (red circles) and corresponding insulin infusion (black solid lines) time profiles of four T1DM (A–D) in a closed-loop setup using spectroscopic glucose analysis of blood microdialysate and MPC algorithm for insulin titration suggestions. Arrows indicate food intake in gram carbohydrates. Dashed horizontal lines indicate target range for intensive care patients 80–110 mg/dl. The solid horizontal line indicates the hypoglycaemic threshold (50 mg/dl).

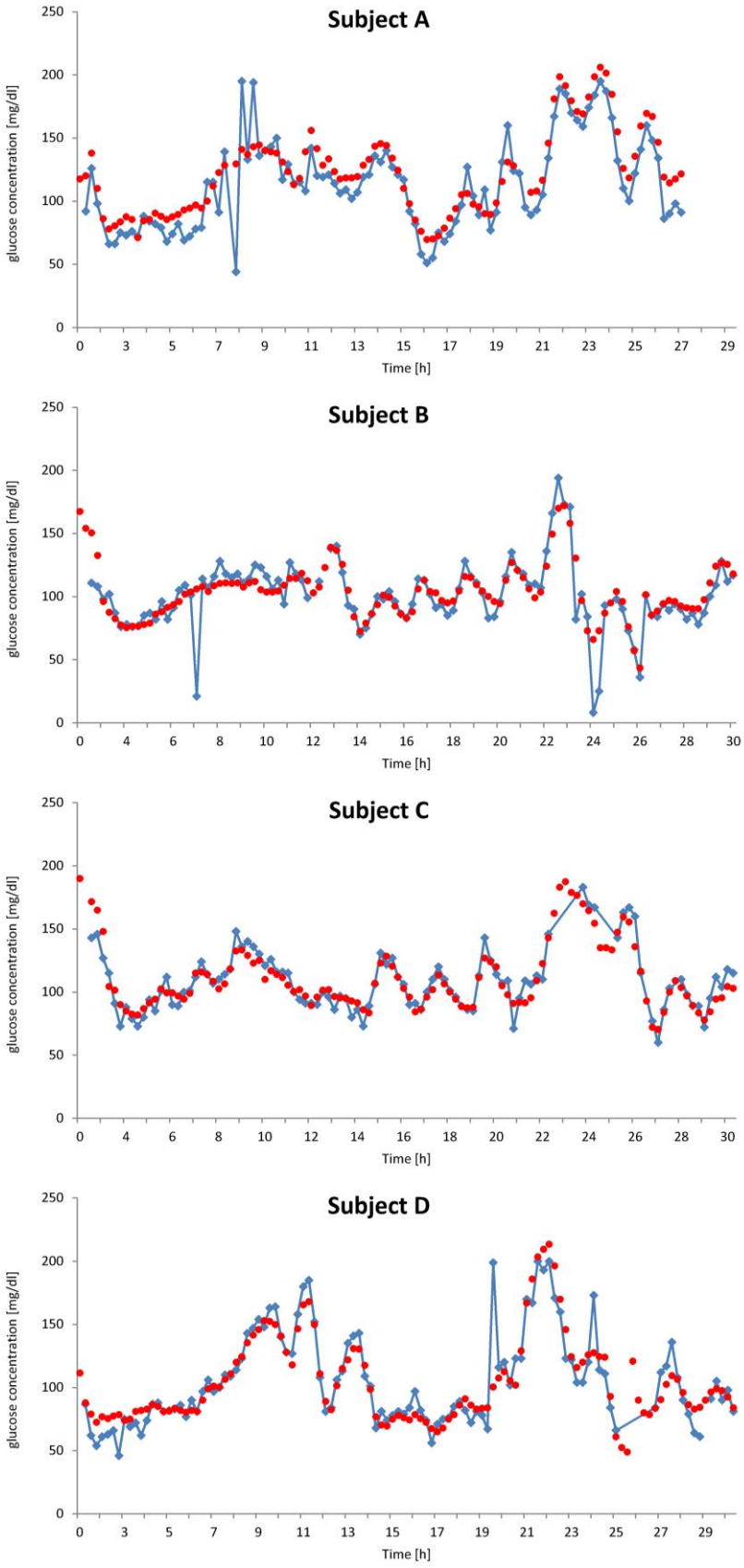

Figure 31: Reference (red) and prospectively one-point calibrated online glucose concentrations as obtained by infrared spectroscopic online glucose sensor

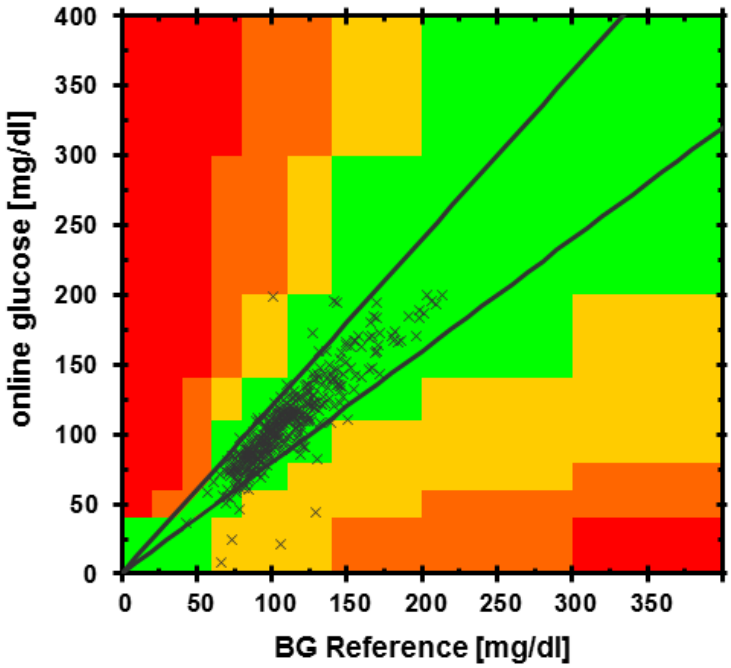

Figure 32: Insulin titration error grid analysis for the glucose monitoring system (PFTMD + infrared spectrometric online glucose sensor) as found in the “closed loop study”

Table 6: Technical performance evaluation summary for the glucose monitoring system (PFTMD + infrared spectrometric online glucose sensor) as found in the “closed loop study”:

SUBJECT [#]	R [-]	MD [mg/dl]	MAD [mg/dl]	MRD [%]	MARD [%]	% PRESS [%]	ISO MET? [%]
A	0.897	-8.91 ± 15.99	14.13 ± 11.59	-7.74 ± 13.22	12.05 ± 9.41	14.39	84.0
B	0.831	-1.81 ± 14.86	8.74 ± 12.13	-2.2 ± 15.71	8.72 ± 13.22	14.24	95.4
C	0.926	1.22 ± 9.06	7.08 ± 5.75	1 ± 8.27	6.59 ± 5.05	8.32	98.1
D	0.913	-0.01 ± 14.95	9.59 ± 11.44	-0.21 ± 14.92	9.48 ± 11.49	13.43	84.5
ALL	**0.89 ± 0.04**	**-2.3 ± 14.5**	**9.8 ± 10.9**	**-2.2 ± 13.8**	**9.2 ± 10.5**	**12.59 ± 2.88**	**90.7**

3.2 Glycaemic control evaluation

Glycaemic control evaluation parameters are presented as mean ± standard deviation hereafter and are summarised per subject in

Table 7.

Min and max BG averaged 61.1 ± 12.5 mg/dl and 195.4 ± 18.4 mg/dl, respectively (min: 43.5 mg/dl; max: 213.5 mg/dl). Overall BG could be maintained at 110.5 ± 29.7 mg/dl for all subjects. Day- and nighttime BG averaged 113.4 ± 31.8 and 103.3 ± 22.4 mg/dl, respectively. The peak postprandial glucose concentration was found to be 152.0 ± 33.0 mg/dl (dinner: 135.4 ± 18.4 mg/dl, snack: 141.3 ± 28.7 mg/dl, breakfast: 192.9 ± 17.5 mg/dl, lunch: 138.6 ± 31.5 mg/dl). The hyperglycaemic index (Vogelzang et al. 2004) averaged 11.9 ± 5.3 mg/dl.

Normoglycaemia (80–110 mg/dl) was established 105 ± 78 min after the start of the trial and could be maintained for 47 ± 12% of the trial duration. Two hypoglycaemic events were observed (43.4, 49.0 mg/dl), whereof the former was due to a human error, which resulted from having entered a too high carbohydrate content of the corresponding meal into the algorithm. Both hypoglycaemic events were immediately treated with IV glucose bolus administration (10 g each).

Figure 33 shows all subject glucose profiles in one graph and thus allows the qualitative assessment of the glycaemic control.

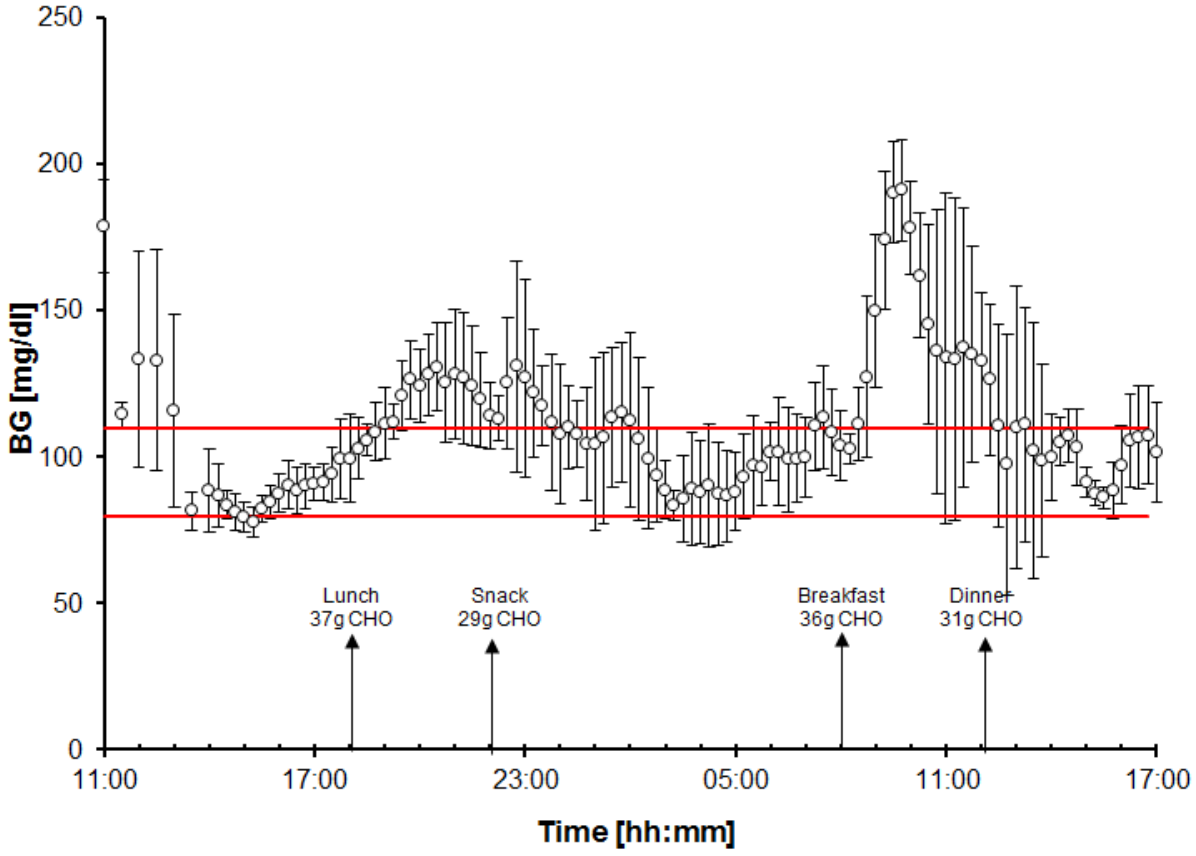

Figure 33: Glucose concentration profiles of all four subjects of the "closed loop study". BG values are shown as means +/- standard deviation.

Table 7: Glycaemic control evaluation parameters as determined in the "closed loop study"

Subject	Min BG	Max BG	Day-time BG	Night-time BG	Mean BG	HGI	Time to Target	Time in Target		Time in extended Target 70 - 145mg/dl	
	[mg/dl]	[mg/dl]	[mg/dl]	[mg/dl]	[mg/dl]	[mg/dl]	[min]	[min]	[%]	[min]	[%]
A	69.5	206	126.2	112.8	122.0	19.3	90	487.9	31.3	1246.7	79.9
B	43.5	172	103.9	103.5	103.8	6.7	157	981	54.5	1601.8	89.0
C	70.5	190	116.6	99.3	111.7	10.8	172.4	989.7	56.9	1505.3	86.5
D	61	213.5	108.6	97.6	105.5	10.8	1.9	781.7	44.2	1468.4	83.0
MEAN	61.1 ± 12.5	195.4 ± 18.4	113.4 ± 31.8	103.3 ± 22.4	110.5 ± 29.7	11.9 ± 5.3	105.3 ± 77.7	810.1 ± 235.3	46.7 ± 11.7	1455.6 ± 150.2	84.6 ± 4

3.3 Clinical analysis

The analysis according to the grading system presented by Chassin and colleagues (L. J. Chassin et al. 2005) revealed that – with respect to the postprandial glucose control (3 h following meal ingestion) – 35.2 and 27.1% of the time was spent in grades A and B, respectively (C: 12%, D: 24.4%, E: 0.6%, F: 0.6%). With respect to the outside-meal glucose control, 22.5 and 63.4% of the time was spent in grades A and B, respectively (C: 2.1%, D: 12.1%, E: 0%, F: 0%). These results are summarised in **Table 8**.

Table 8: Results of a clinical assessment using the grading system developed by Chassin et al (L. J. Chassin et al. 2005)

	Percentage of time spent in Grades A-F						
	A	B	C	D	E	F	total
outside meal	22.5	63.4	2.1	12.1	0.0	0.0	100
post-prandial	35.2	27.1	12.0	24.4	0.6	0.6	100
outside meal and postprandial	27.7	48.6	6.2	17.1	0.2	0.2	100

4. Summary and conclusion[10]

4.1 Study Design

Three subsystems of a closed-loop device for intensive care patients had been developed and performance tested individually in clinical trials involving healthy individuals (Franz Feichtner et al. 2010; H. M. Heise, Damm, Manfred Bodenlenz, et al. 2007b) T1DM patients (H. M. Heise et al. 2008; Roman Hovorka et al. 2010; Roman Hovorka, Valentina Canonico, et al. 2004; Roman Hovorka, L. J. Chassin, et al. 2004) and ICU patients (Johannes Plank, Blaha, Cordingley, et al. 2006; Kulnik et al. 2008) beforehand. In this feasibility trial these components were combined the first time to form a semiautomatic closed-loop device.

Following a safe and stepwise approach this first feasibility trial was performed in type 1 diabetic volunteers before leaping toward the target population. From an ethical point of view it would not have been justifiable to perform this feasibility study in ICU patients using this prototype that has not been evaluated in volunteers, previously. Both, the ethical pretension to test the device in volunteers, as well as the need to simulate high BG excursions implicated to perform this feasibility test in type one diabetic patients. From a technical point of view, neither the vascular body interface nor the glucose sensor were expected to perform differently between ICU patients, T1DM patients, or healthy individuals.

The MPC algorithm was originally developed for and tested in subjects with T1DM12 and was successfully used in the critically ill (Johannes Plank, Blaha, Cordingley, et al. 2006; Kulnik et al. 2008). The present study, therefore, uses the updated controller that has already been proven to work in ICU patients. Given the arguments above and from an ethical point of view, it was thus straightforward to perform this study in T1DM volunteers instead of intensive care patients, allowing to assess the system's safety and performance characteristics under controlled conditions using evaluation parameters, which apply to intensive care patients.

Due to the pilot character of this study and for safety reasons, online glucose concentration values were crosschecked by comparing them to reference glucose values. It would have been one option then to simply let the MPC work with whatever glucose concentration was suggested by the online sensor. However, the results of the algorithm's performance evaluation would

[10] Partly taken and adapted from (Franz Feichtner et al. 2011)

then have been biased by intermittent erroneous input parameters. Therefore, and again for safety reasons, a 20% relative-difference threshold for glucose concentration values was implemented for steering the algorithm.

4.2 Technical performance evaluation

The monitoring part of the system (i.e., the combination of vascular microdialysis and online spectroscopic glucose sensor) performed better than previously evaluated technologies (Weinzimer et al. 2008; Renard et al. 2010) even though 8.8% of all online glucose concentration levels exceeded the critical 20% relative-difference tolerance to the reference plasma glucose concentration. This is a major finding of the study from a technical point of view, which resulted from combining these subcomponents for the first time. The individual errors of the body interface and the spectroscopic online glucose sensor add up to 8.8%, which is satisfactory but leaves space for improvement. However, only four subjects were investigated in our trial.

The ITEGA, as well as the hyperglycaemic index, as useful measures of glucose control in critically ill patients suggest good glucose control, taking 110 mg/dl as the upper range of normal. Compared to other closed-loop studies in T1DM patients using an SC-glucose monitoring approach (Roman Hovorka et al. 2010; Weinzimer et al. 2008), these data suggest a better glucose control performance with respect to mean BG, mean daytime, and overnight BG, time in target range (80–110 mg/dl), and percentage of time spent in grade A and B zones of combined postprandial and outside-meal periods. Compared to closed-loop studies using BG measurement and the MPC algorithm in ICU patients (Johannes Plank, Blaha, Cordingley, et al. 2006; Kulnik et al. 2008) this approach performed comparably with respect to time in target and mean BG. With respect to time in target, a mean overall time in target of about 47% was found. Comparing this result to the work of others is quite difficult as to the different natures of study designs, target ranges, etc. A study by Kovatchev and colleagues (Kovatchev et al. 2010) in T1DM subjects reported that 78% of time spent overnight was within the target range, which was defined as 70–140 mg/dl, whereas in the herein presented study it is defined as 80–110 mg/dl. If however, Kovatchev’s target range is applied to these data (whole trial period), the total time in target increases to 82.8 ± 5.5%, which is a promising result for this first feasibility trial.

4.3 Safety Aspects

Two hypoglycaemic events were recorded. The first (BG = 43.4 mg/dl) was caused by a human error, due to an erroneously too high nutrition entry into the MPC algorithm. The second (BG =

49.0 mg/dl) was just below the hypoglycaemic threshold and also happened in the postprandial period, allowing to conclude that the algorithm reacted rather aggressively on enteral nutrition intake. The glycaemic index of food and mixtures of foods determine the timing, peak and duration of the glycaemic surge. The MPC performance might be improved if it were provided additional information on the glycaemic index of food to take into account the timing, peak, and duration of the glycaemic surge.

Nonetheless, the results of this feasibility study are promising and it is concluded that this approach is worth implementing in an ICU setting, where the MPC algorithm has already proven to work efficiently (Johannes Plank, Blaha, Cordingley, et al. 2006; Kulnik et al. 2008). However, before going into tests within the target population, further technical improvements have to be made and verified with respect to the controller and the monitoring part of the loop. Following the safe and stepwise approach, another series of closed-loop tests must be performed in T1DM subjects, with technical advanced subcomponents before considering first tests in ICU patients.

CHAPTER VIII

Summary, discussion, conclusions and outlook

1. Summary

Driven by the reported medical benefit of (tight) glycaemic control of ICU patients the EC funded project CLINICIP was initialised. CLINICIP aimed to develop a system for closed loop insulin infusion in critically ill patients. This thesis is emedded in CLINICIP and focussed on the development, the technical and clinical evaluation of an extravascular microdialysis based body interface for continuous glucose monitoring. Several approaches were investigated technically and in a risk assessement study in order to find an appropriate design and optimal operating conditions. The final design of the developed body interface features a system for continuous blood withdrawal and a planar flow-through microdialyser that delivers a protein-free blood dialysate. Following a stepwise approach, this body interface was further combined with spectrometric and amperometric online glucose sensors and later on with an algorithm to function as a continuous glucose monitoring and regulation system. The technial and clinical performance evaluations of this system and its subsystems were done in clinical studies in healthy volunteers and type 1 diabetes mellitus patients. Results from these investigations using the extravascular microdialysis approach were published peer-reviewed as first author. The results of a comparison to similar tests using subcutanous micordialysis probes were peer-reviewed published as co-author.

2. Discussion

In this chapter the thesis is discussed from a bird's view perspective. Detailed technical and methodological discussions can be found in the discussion sections of the previous chapters and in the individual publications.

The herein presented body interface is based on extravascular microdialysis, which was developed in the course of a research project. Right at the beginning it was decided to develop a membrane based body interface in order to provide sensors with a protein free matrix and thus protect them from biofouling effects. As discussed in the chapter "Interstitial vs. blood CGM systems" body interfaces based on subcutaneous microdialysis face several drawbacks compared to blood microdialysis systems, including delay time and differences between subcutaneous and plasma glucose concentration that depend on multiple individual and varying patient factors. Therefore, this discussion does not again consider any subcutaneous microdialysis methods.

2.1 Membrane area, perfusion flow and recovery

By nature, probe based MD systems are limited in size (i.e. probe length and diameter) and thus their membrane surface area. A typical membrane for a probe based MD system has an outer diameter of 0.6 mm and a length of max. 30 mm, giving an active diffusion surface of approx. 56 mm². For high recovery rates the probe must be perfused at low perfusion rates (typically ~1 µl/min), which in turn result in dispersion effects, long transport and long lag times. As a result probe based systems are facing a trade-off between recovery rate and lag time.

The herein presented system is based on extravascular microdialysis. The membrane is located outside of the body and by design its surface area is not limited in size and can be optimised together with the perfusion flow rate with respect to maximising the recovery. With this approach high recovery rates of around 100% were achieved at flow rates of about 5 µl/min and an active diffusion surface of 90 mm². The delay time from blood sampling to online glucose signal is approx. 3.5 min.

Literature reports that membranes exposed to blood tend to clot and lead to decreasing recovery over time (compare chapter “Principles of iv-MD”). The herein presented approach features a specially designed membrane (protected intellectual property) with an extremely smooth blood contacting surface. In the in-vivo experiments the recovery remained stable for 30 hours and for 72 hours in in-vitro experiments.

2.2 Discussion on the state of the art

Intravascular microdialysis (iv-MD) probes are difficult to insert into a (peripheral) vessel, when blood streams out the insertion/guidance catheter. Especially longer probes with longer membranes are easily bent during insertion and might become damaged. It is also possible that particles of the fragile membrane are transferred into the vascular system and cause adverse health effects. Furthermore, if the iv-MD probe protrudes from the catheter tip it represents an obstacle in the vessel, it tends to clot and the risk of coagulation/thrombosis increases. This risk is further enhanced when the probe diameter is very large compared to the vessel diameter. Therefore, Rooyackers et al performed their iv-MD study with CMA probes in subjects’ veins with a diameter of more than 3 mm to ensure high enough blood flow around the catheter for sufficient equilibrium across the membrane (O Rooyackers et al. 2013). To reduce the risk of coagulation and catheter/probe clotting heparin-containing perfusate is used. However, it remains questionable if this measure sufficiently reduces the coagulation risk.

The listed aspects are major limitations of iv-MD. It may be concluded that intravenous microdialysis cannot not safely be applied especially in humans having smaller veins (e.g. elderly people, children, women). It might be further concluded that iv-MD using such large microdialysis probes (0.6mm diameter) might even lead to thrombosis because blood is hindered in passing the vein or even blocked.

It can be speculated that this might have been a reason why CMA withdrew its i-View from the market.

In contrast to peripheral iv-MD systems, central iv-MD systems enable to use a large enough membrane area for a high flow rate of microdialysis fluid, still with a high level of equilibration (Christina Blixt et al. 2013).

As described previously two central venous iv-MD systems are currently developed and CE approved. No publications are available on the DIRAMO system. The second central venous system is the EIRUS™ system, which features a specially designed multilumen central venous catheter with a semipermeable microdialysis membrane, that forms part of the outer catheter surface. The device also features a monitor and a biosensor, which requires calibration to plasma glucose concentration.

The results of the recently published EIRUS™ trials are promising in terms of system precision, accuracy and met ISO criterion (Christina Blixt et al. 2013; Schierenbeck et al. 2012; Schierenbeck et al. 2013; Möller et al. 2011). However, there is a major drawback of this approach:

It only works with the EIRUS™ multi-lumen catheter and is thus incompatible with other standard central venous multi-lumen catheters. What's more, two lumens cannot be used as they are reserved for perfusate and dialysate transport.

It is questionable whether physicians would switch to that catheter for the sake of enabling glucose monitoring. It is speculated that the costs for this approach massively exceed the costs of a standard multi-lumen catheter and it is thus questionable whether healthcare providers are willing to pay for that.

2.3 Performance and handling

The developed body interface and the spectrometric online glucose sensor form a highly accurate continuous blood glucose monitoring system with a mean difference of -2.3 ± 14.5 mg/dl and a mean coefficient of correlation of 0.89 ± 0.04. These results are comparable to those obtained in recent iv-MD studies in terms of accuracy and precision (Christina Blixt et al. 2013; Schierenbeck et al. 2012; Schierenbeck et al. 2013; Möller et al. 2011).

This continuous blood glucose monitoring system was also used in a closed-loop feasibility study using a MPC algorithm aiming to establish normo-glycaemia in type 1 diabetic subjects. A mean overall time in extended target range (70-140mg/dl) of about 83% was found, which is comparable or even slightly better than other recent closed loop studies in T1DM subjects (Kovatchev et al. 2010).

The herein presented approach of extravascular microdialysis offers the option to not only monitor glucose, but to monitor all substances of relevance, that can pass the membrane. The body interface was combined with a spectrometric online sensor with the option to measure further analytes such as lactate or urea that might be of interest for intensive care medicine.

2.4 Limitations

Despite the good performance and the promising achieved results of the approach, the developed body interface has several limitations, especially with regard to handling:

1) The inner lumen of the double lumen catheter is tricky to insert into the outer lumen, which is a special venous catheter.

2) The system is fluidically complex and thus not easy to use as it is. Two pumps ensure the continuous blood sampling (one for heparin infusion, one for blood+heparin withdrawal). The third pump drives the microdialysis flow. However this was a research and not a development project. The pumps were computer driven though and worked automatically, but still the fluidic complexity could be minimised. The introduction of short bolus infusions reduced the double lumen catheter's tendency to clot. However, more frequent short bolus infusions could further reduce coagulation problems.

3) Another drawback of this approach is that continuously withdrawing blood using this approach means, that blood is diluted by 50% and online glucose sensors need to be optimised for the reduced dynamic glucose range.

4) Yet another drawback is blood loss. For the investigations performed in this thesis, the continuous withdrawal rate was limited to 2ml/h, which was approved to be safe by medical professionals and would also work in certain ICU patients. However, it would be preferable to have no blood loss at all and to re-infuse blood after a measurement cycle. This is not a difficult task from a technical perspective, but for safety reasons this was not done in these investigations.

The initial requirement to develop a real continuous blood glucose monitor determined the implementation of continuous blood sampling. However all these drawbacks would diminish, if blood was withdrawn intermittently and re-infused thereafter. Such a system for discontinuous blood sampling was presented by Schaller et al. (R Schaller et al. 2009; Roland Schaller, Franz Feichtner, Hans Köhler, Manfred Bodenlenz, Johannes Plank, A. Wutte, Julia K Mader, Martin Ellmerer, Reinhard Hainisch, et al. 2009). It does not need a double lumen catheter, it is fluidically less complex and it does not waste blood.

3. Conclusions and outlook

Independent of the truth beneath the ongoing discussion about tight or safe glycaemic control it can be concluded that glucose control is of lifesafing value for ICU patients. Regarding microdialysis based approaches for glucose monitoring, the following can be summarised and concluded from the previous chapter:

1) Blood glucose rather than ISF glucose monitoring must be preferred
2) Microdialysis is an appropriate measure to protect sensors from biofouling
3) Peripheral intravascular microdialysis did not succeed yet
4) Central intravascular microdialysis shows promissing results but the only system with published data so far only works with a proprietary catheter and it might be too cost intensive
5) Continuous extravascular microdialysis showed promissing results but requires improvement to reduce fluidic complexity and to improve handling
6) Discontiuous extravascular microdialysis might overcome the above drawbacks of extravascular and intravascular microdialysis and might move glucose monitoring for tight glycaemic control to a next level

Recently Zijlstra et al. presented first results of such a discontinuous extravascular microdialysis system at the American Diabetes Association conference in 2012 (Zijlstra et al. 2012). The approach is patented by Künnecke (Künnecke 2010) and is based on a simple Luer connector, which can be connected to any standard central or peripheral venous catheter. This Luer connector features an integrated microdialysis membrane and three fluid channels – one for withdrawing blood and the other two for performing microdialysis. **Figure 34** shows an exemplary embodiment. Blood is withdrawn through the inner lumen (which is formed by the microdialysis membrane), remains inside until the microdialysis process is finished and is flushed back afterwards with an aqueous solution also containing a calibration medium for repeated sensor calibration.

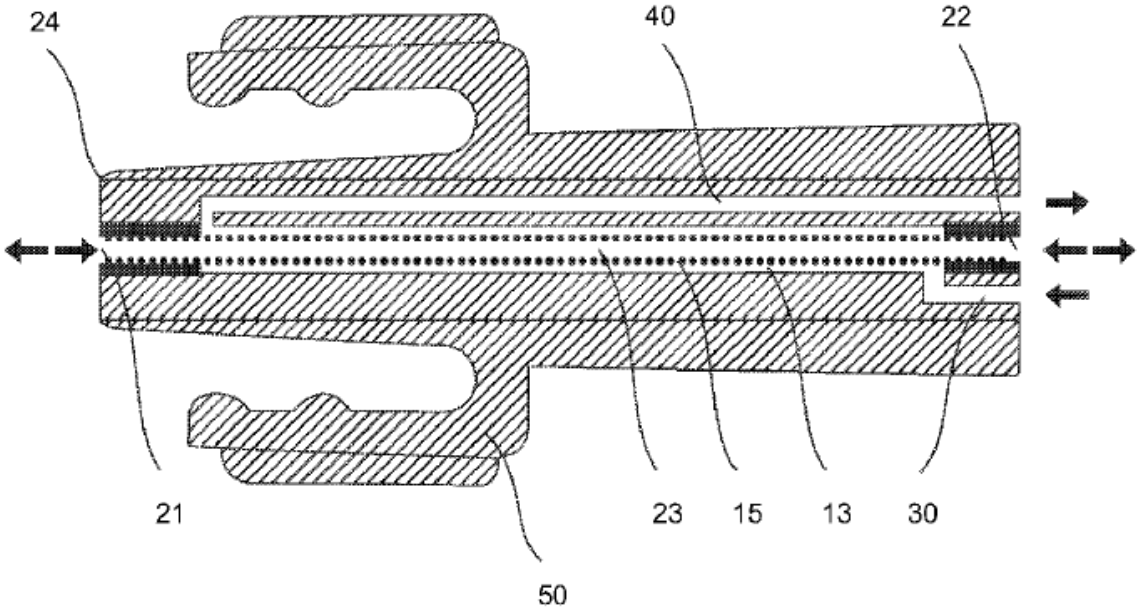

Figure 34: Exemplary embodiment of a next generation miniaturised extravascular microdialysis system.

The preliminary data shown at the ADA suggest that the system had a mean relative deviation of 9.4%. 91.4% of the glucose readings were accurate according to the ISO15197 criteria and 99.3% of the data were in the AB zone of the Clark Error Grid analysis (Zijlstra et al. 2012). However, these data were presented at a conference and are not peer-review published.

This approach is so small that it might be questioned whether the membrane surface is sufficiently large to obtain high recovery rates.

In summary this thesis presents an extravascular microdialysis system that continuously delivers highly recovered dialysate from continuously withdrawn blood. It was combined with online glucose sensors and its clinical evaluation suggests that it performed highly accurate and precisely. It became clear in the discussion section that extravascular microdialysis might outperform intravascular microdialysis. The herein presented discontinuous extravascular microdialysis approach has some limitations which might be overcome by the new miniaturised discontinuous extravascular microdialysis approach.

However, in this thesis extravascular microdialysis was extensively investigated on a research level, the approach presented in this thesis moved forward continuous glucose monitoring for ICU patients and it was a precursor for future extravascular microdialysis systems.

CHAPTER IX

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Author's publications

In the following the author's peer-reviewed and non-peer-reviewed publications, oral and poster presentations are listed. My two peer-reviewed published first-authorships are reprinted thereafter.

JOURNALS

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Microdialysis based device for continuous extravascular monitoring of blood glucose

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Abstract Glycemic control of intensive care patients can be beneficial for this patient group but the continuous determination of their glucose concentration is challenging. Current continuous glucose monitoring systems based on the measurement of interstitial fluid glucose concentration struggle with sensitivity losses, resulting from biofouling or inflammation reactions. Their use as decision support systems for the therapeutic treatment is moreover hampered by physiological time delays as well as gradients in glucose concentration between plasma and interstitial fluid. To overcome these drawbacks, we developed and clinically evaluated a system based on microdialysis of whole blood. Venous blood is heparinised at the tip of a double lumen catheter and pumped through a membrane based microfluidic device where protein-free microdialysate samples are extracted. Glucose recovery as an indicator of long term stability was studied *in vitro* with heparinised bovine blood and remained highly stable for 72 h. Clinical performance was tested in a clinical trial in eight healthy volunteers undergoing an oral glucose tolerance test. Glucose concentrations of the new system and the reference method correlated at a level of 0.96 and their mean relative difference was 1.9±11.2%. Clinical evaluation using Clark's Error Grid analysis revealed that the obtained glucose concentrations were accurate and clinically acceptable in 99.6% of all cases. In conclusion, results of the technical and clinical evaluation suggest that the presented device delivers microdialysate samples suitable for accurate and long term stable continuous glucose monitoring in blood.

Keywords Microdialysis · Sampling · Glucose monitoring · Extravascular · *In vitro* study · Clinical trial

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1 Introduction

Tight glycemic control of critically ill patients was shown to substantially reduce mortality and morbidity in this patient group (van den Berghe et al. 2001) and is associated with substantial reduction in medical care cost (van den Berghe et al. 2006). Thus, the EU funded project CLINICIP (Closed Loop Insulin Infusion for Critically Ill Patients) aimed to develop a *continuous* low-risk monitoring and control device which allows maintaining metabolic control in intensive care patients. Our task in this project was to develop the very first part of such a glycemic control loop, the continuous glucose monitoring system and to evaluate it technically and clinically.

Most current glucose monitoring systems for diabetic (Skyler 2009) and intensive care patients (Kondepati and Heise 2007) are based on sensing in the extra cellular fluid (ECF) of the subcutaneous adipose tissue. However, none of these systems may be used as a decision support system for therapeutic treatment. Additionally, various studies

calculated or measured a physiological time lag between the systemic blood glucose concentration and the glucose concentration of ECF (Regittnig et al. 2003: ~28 min; Roe and Smoller 1998: 0–45 min, average lag 8–10 min; Kulcu et al. 2003: 5 min in average in diabetic subjects). Furthermore, a gradient between interstitial and plasma glucose concentrations was reported, that varied between 20% (Sternberg et al. 1996) and 110% (Bantle and Thomas 1997). Bad correlation between blood and ECF glucose concentration was also found in critically ill children (Vlasselaers et al. 2007) and critically ill patients with severe traumatic brain injuries (Lourido et al. 2002). From a therapeutic point of view it is therefore obvious not to access ECF but blood for continuous glucose monitoring in intensive care patients, moreover, because blood access is available in these patients anyway.

Years before the van den Berghe study (van den Berghe et al. 2001) initialised the debate about tight glycemic control of intensive care patients, Stjernstrom and co-workers measured different metabolites, including glucose, in intensive care patients accessing the patients intravenously. They first used a technique of intravenous microdialysis (Stjernstrom et al. 1993), but only a few publications arose since then repeating this technique in humans (O'Connell et al. 1996; Patsalos et al. 1996; Paez and Hernandez 1997; Castejon et al. 1999; Costa et al. 1999; Dizdar et al. 1999a, b; Elshoff and Laer 2005). A reason for this small number of studies might be the risks associated with implanting a fragile membrane into the vascular system as such probes were hand-made and non-commercially available. What's more, decreasing microdialysis efficiencies of membranes were repeatedly reported to occur *in vitro* and *in vivo* shortly after probe implantation (Yokel et al. 1992; Chen and Steger 1993; Yang et al. 1997; Verbeeck 2000) even in heparinised blood (Sauernheimer et al. 1994), which might be due to clot formation around intravenously implanted probes (De Lange et al. 2000). However, in the meantime CMA Microdialysis AB has launched a product for intravenous microdialysis, the IView, but there are no published data available yet.

As solution for the limitations mentioned above we decided to develop a device that *continuously* withdraws heparinised blood from a peripheral vein to an *ex vivo* microdialyser. Thereby we are able to combine various advantages of current state-of-the-art techniques. Direct access to blood enables to *continuously* monitor the systemic glucose concentration and performing microdialysis outside the patient is safer than placing a fragile membrane into the vascular system. To overcome the described drawback of decreasing efficiencies of microdialysis membranes that are exposed to blood, we developed also a new membrane with a very "smooth" blood contacting surface, onto which blood clot formations are very unlikely to adhere. The membrane is thus very long-term stable with respect to diffusive transport properties.

In this paper, we report on the design, the technical and the clinical validation of this microdialysis based device for continuous extravascular monitoring of blood glucose.

2 Materials and methods

2.1 Continuous blood sampling

A double lumen catheter (DLC; mtb GmbH, Lonsee, Germany) is used to continuously withdraw blood from a peripheral vein using a Gilson Minipuls MP3 peristaltic pump (Gilson, Cedex, France). To prevent coagulation a NaCl-Heparin solution (50 IU/ml) is delivered to the tip of the catheter via the outer lumen of the DLC using another Gilson Minipuls 3 peristaltic pump at a flow rate of 2 ml/h. Anti-coagulated blood is withdrawn simultaneously through the inner lumen of the DLC at a flow rate of 4 ml/h. This well-established technique is described in detail elsewhere (Weller et al. 1960) and has been used in blood sampling and blood glucose analysers such as the Biostator and the Glucostator, respectively. Anti-coagulated blood is pumped through the planar flow-through microdialysis (PFTMD) device and is collected in 30 µl samples for glucose analysis. Pumps are PC-controlled using Lab VIEW® 7.0 software on a notebook and a NI 9263 4-Channel 16-Bit Analog Voltage Output Module (all from National Instruments, Inc., Austin, TX, USA). Backflux of potentially contaminated blood from the waste compartment is prevented by using peristaltic pumps unidirectionally.

2.2 Microdialysis

Microdialysis (MD) is a technique used to extract components of the ECF via a semi-permeable membrane, which goes back to the pioneering work of Ungerstedt (Ungerstedt 1991). Molecules below the membrane's molecular weight cut-off diffuse through the membrane to an analyte-free aqueous solution (perfusate), which is pumped through the microdialysis device. The analyte concentration reached in the aqueous solution (dialysate) is related to the concentration in the extra-cellular fluid and depends on parameters including flow rate and temperature of both liquids, the analyte's molecular weight, its charge and the membrane's surface area. Important to know is that analyte concentrations in microdialysis samples do not fully equilibrate with the surrounding tissue unless inapplicably low perfusion flow rates (~1 µl/min) are chosen (Rosdahl et al. 1998; Ekberg et al. 2005). Thus MD data always require calibration procedures.

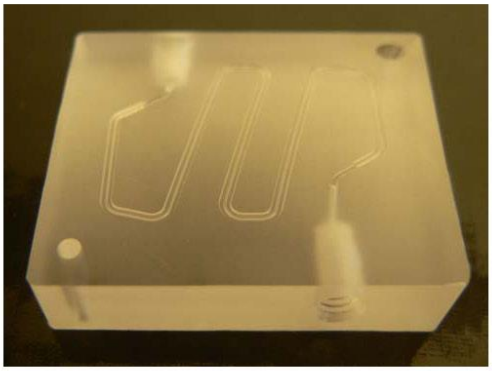

Fig. 1 One of two microfluidic plates with engraved microfluidic channels (8 µl): Perfusate or blood is connected to the plate via HPLC screw connectors. A semipermeable membrane is sandwiched between two of these plates. Analytes below the membrane's molecular weight cut off diffuse from one side of the plate to the other. Analyte enriched perfusate (=dialysate) is collected for glucose analysis

2.3 The planar flow-through microdialysis device (PFTMD)

The PFTMD consists of two polycarbonate plates (37×33×8 mm, Makrolon Rx-1805 from Bayer AG, Leverkusen, Germany) that sandwich a semi-permeable membrane in-between (Fig. 1). On the plate's surfaces meander-like microfluidic channels are engraved with a volume of 8 µl each. The first plate is connected to the DLC with TYGON® tubing (S-50-HL, ID=0.25 mm, OD=0.75 mm, l=1,500 mm, Saint-Gobain Performance Plastics, Beaverton, France) using re-usable custom made HPLC screw-connectors and perfused with heparinised blood. The other plate is connected to a 5% Mannitol solution (Fresenius Kabi, Graz, Austria) with TYGON® tubing (R-3606, ID=0.19 mm, OD=2.01 mm) and is perfused countercurrently.

The PFTMD is a multi-use device. Its polycarbonate plates meet biocompatibility criteria according to ISO 10993-1 standard. Plates and connectors were steam-sterilised at 121°C for 20 min before each experiment. Tubing and membrane are single-use only and were EtO sterilised before use. A schematic illustration of the complete system including the continuous blood sampling system is shown in Fig. 2.

2.4 Membrane

Custom made high-porous hydrophilic PAES flat sheet membranes (Gambro, Hechingen, Germany, molecular weight cut-off=10 kDa, thickness=60 µm, liquid permeability=2 3×10^{-4} cm³/(cm² sec bar)) were prepared by phase separation. A polymer solution was formed by dissolving polyethersulfone (Ultrason 6020, BASF, Ludwigshafen, Germany) and polyvinylpyrrolidone (K30 and K90, BASF) in N-Methylpyrrolidone (NMP). The viscosity of the resulting polymer solution was 5,000 mPa·s. The final degassed homogeneous polymer solution was casted onto a smooth glass plate using an Erichsen Coatmaster 5097 MC-I and a coating knife (gap height 100 µm) at a casting speed of 12.5 mm/s at a temperature of 50°C. The glass plates were immediately immersed into a coagulation bath containing a mixture of NMP and water. The membranes formed were washed with water and dried at 60°C.

The custom made membrane combines high diffusive and controlled convective transport characteristics. Its selective layer is on the blood contacting side with selective pore diameters between 2 10 nm, building an extremely "smooth" blood contacting surface (compare Fig. 3), which reduces the probability of cell and protein adhesion to the membrane surface and thus prevents a loss in dialysis efficiency.

2.5 Pre-clinical experiments

Five PFTMDs were tested *in vitro* in 72 h experiments to find optimum operating conditions with respect to achieve high relative recovery while having short transport delay times. Tests were performed using anticoagulated, glycolysis-inhibited and temperature controlled (37°C) bovine blood as test matrix (anticoagulation: 500 mg potassium oxalate per 1,000 ml blood, glycolysis-inhibition: 750 mg sodium fluoride per 1,000 ml blood) and 5% Mannitol as perfusate. Bovine blood flow rate was fixed at 4 ml/hour, whereas perfusate flow rate was varied between 2, 3, 5 and 10 µl/min.

Reference blood samples (100 µl) were taken directly from the blood pool in hourly intervals. Continuously withdrawn blood samples were collected at the outflow of the PFTMD in 100 µl fractions. Both were centrifuged and supernatant plasma was collected for glucose analysis. Dialysate samples were collected at the dialysate outflow of the PFTMD in 25 µl fractions. All samples were frozen at −80°C for subsequent glucose analysis with a Roche Cobas Mira analyser using Cobas Gluco-quant and Glucose/Hexokinase (Roche Diagnostics GmbH, Mannheim, Germany).

2.6 Clinical investigations

After optimal operating conditions were found in the pre-clinical experiments a technical and clinical evaluation of the PFTMD was performed in a 12 h open mono-centre clinical feasibility trial in eight healthy, non-diabetic volunteers (7 males, 1 female; age: 28.9±3.5 years; BMI: 25.1±1.5 kg/m²). The study was performed according to

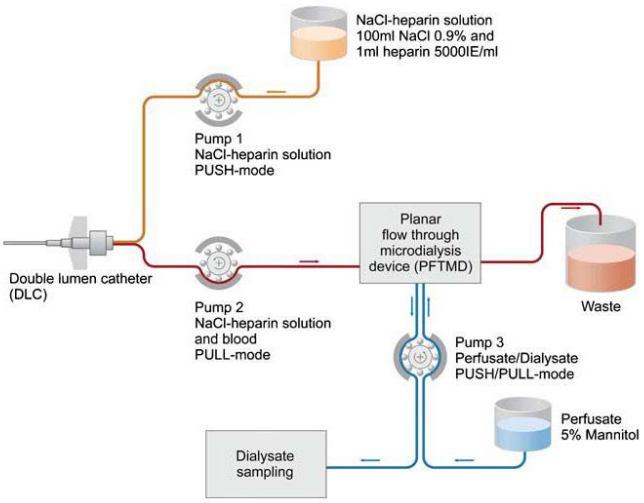

Fig. 2 Schematic illustration of the microdialysis based device for continuous extravascular monitoring of blood glucose. A NaCl heparin solution is pumped to the tip of a double lumen catheter and anti-coagulated blood is continuously withdrawn and pumped to a planar flow through microdialysis device that sandwiches a membrane between two microfluidic plates in a clamp-device. 5% Mannitol as perfusate is delivered to the second microfluidic plate to obtain protein-free dialysate for analysis

Good Clinical Practice (GCP) guidelines at the Clinical Research Centre located at the Medical University of Graz. Ethical approval was obtained from the local ethical committee. Signed informed consent was obtained from each subject before any trial related activities.

2.6.1 Study protocol

Subjects arrived in the morning of the trial in a fasting condition. A peripheral 20 Gauge venous catheter (CODAN pvb Medical GmbH, Lensahn, Germany) and the DLC were applied at the Vena mediana cubiti of the right and the left arm of the subjects for reference and continuous blood sampling, respectively. The DLC was connected to the PFTMD and continuous blood sampling was performed as described above. After this setup procedure the study protocol started (sampling interval: 30 min).

Five hours after the trial start an Oral Glucose Tolerance Test (OGTT) was performed. 75 g of glucose were dissolved in 250 ml of water and were given orally to the subjects. After

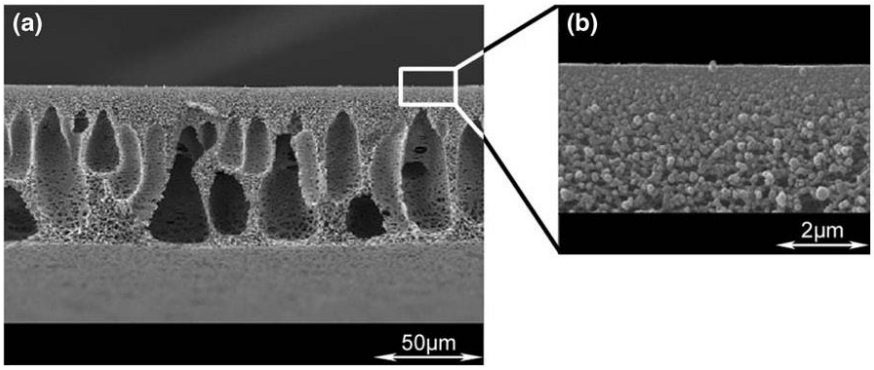

Fig. 3 Scanning Electron Micrographs of the cross section of a hydrophilic microdialysis membrane: (**a**) Whole cross section of the flat sheet membrane (Magnification: 600). (**b**) Active separation layer (Magnification: 20.000). The cross section of the flat sheet membrane in (**a**) clearly allows to identify the novel 3 layer structure: the selective layer on top shows a narrow pore size distribution. It is responsible for the separation of different molecules based on size exclusion (thickness 0.1–0.5 µm, blood contacting side). The support layer on the bottom has a more open membrane structure, with stabilising but no sieving function (perfusate contacting side). In between, the finger-type layer has a very open structure, which gives additional mechanical stability. Due to the high void fraction of this layer the diffusive resistance in this part is nearly identical to the one in water

this glucose bolus 3 h of highly dynamic glucose levels were expected (OGTT period, sampling interval: 15 min). Then a 4-h post-OGTT period followed (sampling interval: 30 min). The trial ended after 12 h.

2.6.2 Sampling

Reference blood samples (REF, 100 µl) were taken from the reference catheter and continuously withdrawn blood samples (CON, 100 µl) were collected at the outflow of the PFTMD. Both samples were centrifuged and supernatant plasma was collected. Microdialysate samples (DIA) were collected for 15 min to get enough sample volume for glucose analysis and were vortexed afterwards. Thus, DIA samples reflect a time integrated glucose concentration. REF and CON samples were taken in the middle of each DIA sampling period. All samples were immediately analysed for glucose concentration using a Beckman glucose analyser.

Additionally, three blood samples were taken from the reference catheter for determining the activated partial thromboplastin time (APTT) at 0 h, 5 h and 12 h in order to verify the absence of any significant patient heparinisation.

2.7 Data analysis

Technical evaluation and data analysis was performed according to Wentholt et al. (2008). The evaluation criteria include relative recovery levels, Bland and Altman analysis, Pearson's coefficient of correlation (R), absolute (AD) and relative differences (RD), mean absolute relative difference (MARD) and mean relative difference (MRD), which is also called the system error (SE) and %PRESS (Lodwig and Heinemann 2003). The latter is very sensitive on outliers and can be calculated according to (1). ISO criterion is met if the system's glucose concentration is within ±15 mg/dl or within 20% of the reference glucose concentration, for glucose concentrations <75 mg/dl and ≥75 mg/dl, respectively. Evaluation was performed on REF and CON glucose data to obtain information about heparin-blood-mixing ratio. REF and DIA data were splined on minute basis and cross-correlated in a ±20 min time window to determine transport delay time which was assumed to be found most likely at the maximum occurring coefficient of correlation. DIA data were prospectively one-point calibrated using the first valid DIA and corresponding REF sample.

Clinical evaluation included Clark Error Grid analysis (Clarke et al. 1987) and Insulin Titration Error Grid Analysis (Ellmerer et al. 2006). Technical performance evaluation was performed using time-delay corrected and prospectively one-point calibrated DIA samples. Clinical performance evaluation was performed using not delay-time corrected DIA data, as they would also not be time corrected in a clinical setting.

Data analysis was performed using Matlab (The MathWorks, Inc., Natick, MA, USA) and Microsoft Excel (Microsoft, Inc., Redmond, WA, USA).

$$\%PRESS = \sqrt{\frac{\sum_{i=1}^{N}\left([Glucose_{DIA}] - [Glucose_{REF}]\right)^2}{\sum_{i=1}^{N}[Glucose_{REF}]^2}} \quad (1)$$

3 Results

3.1 Pre-clinical evaluation

In vitro investigation in bovine blood revealed that the mean relative glucose recovery level (calculated according to (2)) remained stable at a level of around 100% for 72 h at perfusate flows between 2–5 µl/min and a blood flow of 4 ml/min. Detailed results are given in Table 1.

$$recovery = \frac{[Glucose_{DIA}]}{[Glucose_{REF}]} \quad (2)$$

Relative recovery of 100.4±4.2% was achieved at a perfusate flow of 5 µl/min. This flow is regarded as the optimal perfusion flow rate combining complete relative recovery at a perfusion rate yielding in acceptable low system transport delay time. Thus, in the following clinical investigation 5 µl/min was chosen as perfusion flow rate.

3.2 Clinical study—Technical performance evaluation

Blood-heparin withdrawal was monitored in all eight subjects and was found to be 66.9±5.6 µl/min. The system's mean delay time due to blood and dialysate transport was calculated as described above and found to be 10.5 min. In total 240 reference blood samples were taken

Table 1 Relative recovery levels at different perfusate flow rates in 72 h *in vitro* MD experiments using five PFTMD devices, custom made MD membranes and heparinised bovine blood at a flow of 4 ml/h

Perfusate flow [µl/min]	Relative recovery [%]
2	103.6±4.3
3	102.6±2.1
5	100.4±4.2
10	82.7±6.9

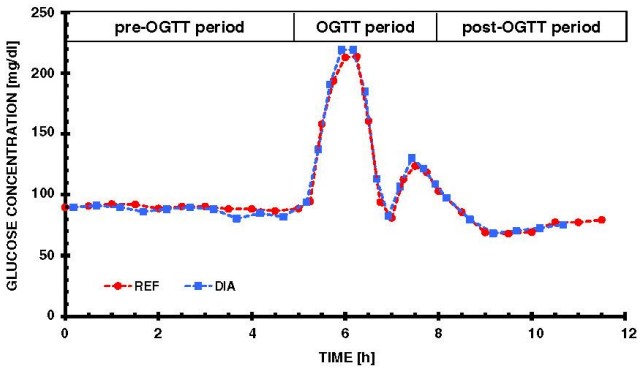

Fig. 4 Exemplary glucose time-profile showing reference (REF, circles) and prospectively one-point calibrated, transport-time-delay-corrected glucose concentration of the microdialysis based device (DIA, squares) for continuous extravascular monitoring of blood glucose concentration during a 12 h feasibility trial in a healthy volunteer undergoing an oral glucose tolerance test (OGTT)

(30 per subject) in a glucose range between 53.0 and 213.7 mg/dl (<80 mg/dl: 31 samples, 80–120 mg/dl: 139 samples, >120 mg/dl: 69 samples). 227 corresponding DIA samples were successfully collected and analysed. In total, 13 DIA samples (5.4%) were not taken due to malfunction of the system, mostly as a result of catheter occlusion.

Figure 4 shows exemplary glucose concentration profiles derived from the reference (REF, circles) and the microdialysis based system (DIA, squares). Microdialysis samples were prospectively one-point calibrated to the first reference blood glucose concentration and their glucose concentrations are corrected by the calculated lag time.

Technical data evaluation was performed according to Wentholt et al. (2008) for all eight subjects accordingly (Table 2). Mean coefficient of correlation (CORR) was found to be 0.96±0.042. Mean difference (MD) was 2.1± 12.1 mg/dl. Mean absolute difference (MAD) was 8.7± 8.6 mg/dl. Mean relative difference (MRD) was 1.9± 11.2%. Mean absolute relative difference (MARD) was 8.4±7.7%. %PRESS parameter was calculated as 10.3± 5.2%. ISO criterion (ISO MET?) was met in 91.6% of all cases. Four out of eight trials were performed with 100% success rate (ISO criterion met).

A Bland and Altman analysis on transport-time-delay-corrected DIA and REF data is provided in Fig. 5.

3.3 Clinical study—Clinical performance evaluation

The Clark Error Grid analysis (EGA, Clarke et al. 1987) is a standard clinical evaluation method classifying data pairs of a new glucose sensing method and a reference method into five zones with different levels of clinical acceptability (A: accurate, B: acceptable, C, D and E: not acceptable).

Table 2 Summary of the technical performance evaluation of the 12-h clinical trials performed in eight healthy volunteers undergoing an oral glucose tolerance test

Subject [#]	CORR [–]	MD [mg/dl]	MAD [mg/dl]	MRD [%]	MARD [%]	%PRESS [%]	ISO MET? [Yes]
1	0.876	6.6±18.2	13.4±13.8	6.7±13.4	11.4±9.5	15.3	21 of 25
2	0.935	5.2±14.8	11.3±10.6	4.0±15.7	11.6±11.0	14.3	21 of 26
3	0.989	−6.5±3.4	6.5±3.4	−6.4±3.1	6.5±3.1	7.1	30 of 30
4	0.936	15.9±10.6	17.0±8.7	16.3±8.9	17.1±7.3	17.8	20 of 27
5	0.995	0.9±4.6	3.6±2.9	0.6±4.3	3.4±2.6	3.9	28 of 28
6	0.984	−6.8±4.6	7.1±4.2	−6.9±4.3	7.2±3.7	8.2	30 of 30
7	0.971	7.5±8.5	8.1±7.9	6.8±7.6	7.6±6.9	11.2	27 of 30
8	0.994	−3.4±3.8	4.0±3.2	−3.6±4.0	4.0±3.5	4.3	30 of 30
ALL	0.960±0.042	2.1±12.1	8.7±8.6	1.9±11.2	8.4±7.7	10.3±5.2	207 of 226

Prospectively one-point calibrated, transport-time-delay-corrected microdialysate samples are evaluated against reference blood samples with respect to their glucose concentrations. Evaluation parameters include the Pearson's coefficient of correlation (CORR), mean difference (MD), mean absolute difference (MAD), mean relative difference (MRD), mean absolute relative difference (MARD), %PRESS and ISO-criteria (ISO MET?). Evaluation parameters are given as means ± standard deviation for each subject and for all subjects in summary

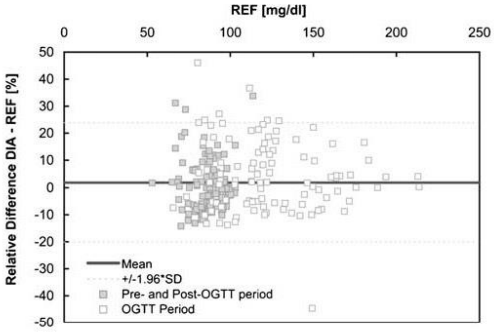

Fig. 5 Bland and Altman plot showing relative differences (y-axis) of transport-time-delay-corrected DIA and REF data plotted against REF data (x-axis). Data pairs are shown as full squares during periods of relatively stable glucose concentrations (pre- and post-OGTT period: 0–5 h and 8–12 h, respectively) and as open squares during periods with pronounced glucose excursions (OGTT-period, 5–8 h)

For this evaluation the DIA data were again prospectively one-point calibrated as described before but were not delay-time corrected, as they would also not be in a clinical setting. The EGA analysis revealed that 85.9% (n=195) and 13.7% (n=31) were in zones A and B, respectively. One glucose sample (0.4%) was found to be in zone D. The EGA plot is depicted in Fig. 6, left.

Another clinical evaluation method for glucose monitoring systems is the insulin titration error grid analysis (ITEGA). It was developed for intensive care patients undergoing intensive insulin therapy (Ellmerer et al. 2006). Four zones in a scatter-plot represent different degrees of accurate therapeutic treatment assuming the therapy decision was based on the actual glucose measurement (appropriate treatment (a), unacceptable violation (b), major violation (c), life threatening violation (d)). 99.1% (=225) of all 227 microdialysate (DIA) glucose concentrations would have led to appropriate treatment, whereas 0.9% (n=2) would have led to an unacceptable violation in insulin therapy (compare Fig. 6, right).

3.4 Safety endpoints

All eight subjects successfully finished the trial at the foreseen trial end. No trial related adverse event occurred. As heparin was used to anti-coagulate blood for continuous withdrawal within a double lumen catheter, we for safety reasons monitored the subject's activated partial thromboplastin time (APTT) as a measure of systemic heparinisation. Three APTT levels were measured per subject and none of them significantly increased during the trial (p>0.05).

4 Discussion and conclusion

This study presents a novel microdialysis based device for continuous extravascular monitoring of blood glucose. A planar flow-through microdialyser was designed and technically evaluated in combination with a continuous blood sampling system in 72 h *in vitro* investigations. In contrast to reported recovery degradation following probe implantation into a blood vessel (Yokel et al. 1992; Chen and Steger 1993; Yang et al. 1997; Verbeeck 2000) we can report that the glucose recovery levels remained stable in our investigations using a newly designed membrane, letting us conclude that the membrane is superior to those

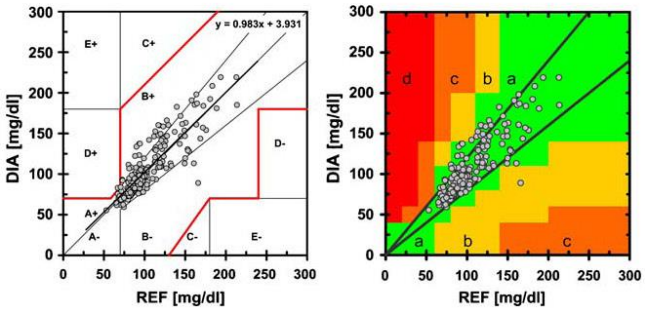

Fig. 6 Left: Clark Error Grid Analysis (EGA) comparing prospectively one-point calibrated microdialysate (DIA) with reference blood (REF) sample's glucose concentrations. 99.6% of all 227 data pairs are found in accurate and clinically acceptable zones A and B. One data pair is found in clinically unacceptable zone D. Right: Insulin Titration Error Grid Analysis (ITEGA) using the same data. 99.1% of all 227 data pairs are found in the (**a**)-zone, representing the "appropriate treatment" zone. Two are found in the (**b**)-zone "unacceptable violation"

presented in the literature with respect to recovery stability when exposed to heparinised blood.

After optimising the operating conditions, a clinical study was performed in eight healthy subjects in order to evaluate the system technically and clinically. The study was finished successfully in all subjects. A clinical performance evaluation showed that the device is able to deliver glucose information that in 99.6% of all cases was found to be accurate and clinically acceptable.

Also a technical performance evaluation was done considering Bland and Altman analysis and state of the art estimate parameters including absolute and relative differences, mean absolute relative difference, the system error %PRESS and ISO criterion. The latter was met in four out of eight experiments and we identified that 78% of all ISO deviations occurred during the OGTT period, thus during times of pronounced glucose changes. We could not find any correlation between met or unmet ISO criterion and the length or the amplitude of glucose concentration during the OGTT period, but it might be worth considering here that the performed clinical and technical evaluation is based on 15 min-time-integrated glucose concentrations of dialysate samples that were compared to spot measurements of reference blood glucose concentration. Dialysate samples thus represent a 15 min glucose average, which especially in periods with pronounced glucose changes (OGTT period) can result in relatively high discrepancies between glucose concentrations of reference and dialysate blood samples. All other evaluated clinical and technical performance parameters, let us conclude that the developed system stably delivers a continuous dialysate-matrix upon which the estimation of blood glucose concentrations can be performed precisely and highly accurate. However, we believe that the precision of the system could even be further improved, e.g. by using online glucose sensors instead of offline glucose analysis of time-integrated dialysate samples and by incorporating an online recovery monitor such as suggested by Schaupp et al. (1999) or Yokel et al. (1992).

The current limitations of the system include blood loss, time delay and the lack of an integrated online glucose sensor. The former is inherent and cannot be diminished in our approach, but the blood withdrawal rate of 2 ml/h was chosen according to specifications of the DLC-manufacturer and approved to be safe by intensive care professionals. However, it can be speculated that blood waste might be reduced by further downscaling system dimensions, by re-infusing the analysed blood or by integrating the analytics for other relevant blood gases and metabolites.

The time delay of our system currently is 10.5 min, therefore therapy decision would be based on 'old' glucose data. However, it was shown by the EGA and ITEGA analysis that this time delay would not result in unappropriate treatment. Moreover, the delay time could be improved by increasing perfusate flow or by introducing low-volume online sensors.

The system has currently not been combined with online glucose sensors and thus allows only a limited number of samples to be analysed when used with offline laboratory glucose meters such as the herein used Beckman glucose analyser, which requires at least 10 µl per sample. Integration of low volume online sensors (e.g. 0.5 µl) such as presented by Schaller et al. (Schaller et al. 2009) is feasible, obtaining highly resolved glucose signals using a 5 µl/min perfusate flow and will be investigated in future studies where aspects of longterm *in vivo* stability will also be addressed.

In summary, we have successfully developed and tested a device that is able to continuously deliver dialysate that is extracted from whole blood outside the human body for the purpose of continuous glucose monitoring. The next step towards an *automated* continuous glucose monitoring system is to integrate online glucose sensors into the herein presented microdevice.

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Competing interests statement The authors declare to have no competing interests.

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ORIGINAL ARTICLE

A Stepwise Approach toward Closed-Loop Blood Glucose Control for Intensive Care Unit Patients: Results from a Feasibility Study in Type 1 Diabetic Subjects Using Vascular Microdialysis with Infrared Spectrometry and a Model Predictive Control Algorithm

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Abstract

Background:

Glycemic control can reduce the mortality and morbidity of intensive care patients. The CLINICIP (closed-loop insulin infusion for critically ill patients) project aimed to develop a closed-loop control system for this patient group. Following a stepwise approach, we combined three independently tested subparts to form a semiautomatic closed-loop system and evaluated it with respect to safety and performance aspects by testing it in subjects with type 1 diabetes mellitus (T1DM) in a first feasibility trial.

Methods:

Vascular microdialysis, a multianalyte infrared spectroscopic glucose sensor, and a standard insulin infusion pump controlled by an adaptive model predictive control (MPC) algorithm were combined to form a closed-loop device, which was evaluated in four T1DM subjects during 30-hour feasibility studies. The aim was to maintain blood glucose concentration in the target range between 80 and 110 mg/dl.

Results:

Mean plasma glucose concentration was 110.5 ± 29.7 mg/dl. The MPC managed to establish normoglycemia within 105 ± 78 minutes after trial start and managed to maintain glucose concentration within the target range for 47% of the time. The hyperglycemic index averaged to 11.9 ± 5.3 mg/dl.

Conclusion:

Data of the feasibility trial illustrate the device being effective in controlling glycemia in T1DM subjects. However, the monitoring part of the loop must be improved with respect to accuracy and precision before testing the system in the target population.

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Abbreviations: (BG) blood glucose, (CHO) carbohydrate, (CLINICIP) closed-loop insulin infusion for critically ill patients, (ICU) intensive care unit, (ITEGA) insulin titration error grid analysis, (IV) intravenous, (MPC) model predictive control, (SC) subcutaneous, (T1DM) type 1 diabetes mellitus

Keywords: CLINICIP, MPC algorithm, spectroscopic glucose sensor, tight glycemic control, vascular microdialysis

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Introduction

Establishing glycemic control can be beneficial for intensive care patients.[1] Glycemic control devices that use the subcutaneous (SC) monitoring route encounter performance difficulties as SC-glucose levels face physiological lag times and low correlation compared to plasma glucose in certain patient groups.[2,3] We therefore developed a vascular-based monitoring and glycemic control device, comprising a body interface for the continuous extraction of blood dialysate,[4] an infrared spectroscopic glucose sensor,[5,6] and a model predictive control (MPC) algorithm[7,8] that generates advice with respect to insulin infusion rates. Following a stepwise approach, these components were performance-tested separately and individually in healthy individuals, patients with type 1 diabetes mellitus (T1DM), and intensive care unit (ICU) patients. In this feasibility trial, we combined these components for the first time to form a closed-loop device. Before approaching the target population, we performed this feasibility trial in T1DM volunteers, evaluating safety and performance issues while trying to establish normoglycemia semiautomatically.

Research Design and Methods

The 30-hour feasibility studies were performed in four T1DM volunteers (body mass index: 25.8 ± 6.3 kg/m²; age: 31 ± 8 years; three male; diabetes history: 11.4 ± 9.0 years; hemoglobin A1c: 7.2 ± 0.8%) in a supine position. Each subject received four standardized meals (dinner: 6 p.m., snack: 10 p.m., breakfast: 8 a.m., lunch: 12 p.m.) sized 37, 29, 36, and 31 g carbohydrates (CHO), respectively.

Peripheral venous blood was continuously withdrawn from a standard intravenous (IV) line at 2 ml/hour and pumped to an extracorporeal membrane-based microdialyzer by which a protein-free matrix was generated. Dialysate was analyzed further for glucose concentrations using an online infrared spectrometric sensor, with the option to measure further analytes such as lactate, urea, and pCO_2 of interest for intensive care medicine. A similar spectrometric system has been successfully demonstrated for plasma glucose monitoring.[9] For our system, average sensor readings were obtained at 5-minute intervals. Every 15 minutes, sensor-derived glucose mean concentration values were entered into a laptop computer running the MPC algorithm.

In the present study, we used a control algorithm based on a MPC paradigm.[10] The algorithm is based on a model of glucose regulation in T1DM described in detail by Hovorka and colleagues.[11] The MPC controller was originally developed and tested in subjects with T1DM[12] and a modified version was used in the critically ill.[8,13] The present study uses the updated controller in a population for which it was originally developed. According to the Leuven insulin titration guideline,[1] the algorithm was initialized to 80 and 110 mg/dl as lower and upper limits of normoglycemia. The suggested insulin dosage was then administered by a standard IV-insulin infusion pump. For safety reasons, only online sensor values within ±20% of reference plasma glucose concentrations were used as input for the algorithm. Otherwise, venous plasma glucose concentrations measured with a Beckman glucose analyzer were used [hypoglycemia: blood glucose (BG) <50 mg/dl].

The study received approval from the Ethics Committee of the Medical University of Graz.

Results

Figure 1 shows the subjects' BG concentration profiles and corresponding insulin administration rates. Glycemic control parameters are presented hereafter as mean ± standard deviation. BG could be maintained at a mean BG of 110.5 ± 29.7 mg/dl for all subjects. Day- and nighttime BG averaged 113.4 ± 31.8 and 103.3 ± 22.4 mg/dl, respectively. The peak postprandial glucose concentration was found to be 152.0 ± 33.0 mg/dl (dinner: 135.4 ± 18.4 mg/dl, snack: 141.3 ± 28.7 mg/dl, breakfast: 192.9 ± 17.5 mg/dl, lunch: 138.6 ± 31.5 mg/dl). The hyperglycemic index[14] averaged 11.9 ± 5.3 mg/dl. An analysis according to the grading system presented by Chassin and colleagues[15] revealed that—with respect to the postprandial glucose control (3 h following meal ingestion)—35.2 and 27.1% of the time was spent in grades A and B, respectively (C: 12%, D: 24.4%, E: 0.6%, F: 0.6%). With respect to the outside-meal glucose control, 22.5 and 63.4% of the time was spent in grades A and B, respectively (C: 2.1%, D: 12.1%, E: 0%, F: 0%).

Normoglycemia (80–110 mg/dl) was established 105 ± 78 min after the start of the trial and could be maintained for 47 ± 12% of the trial duration. Two hypoglycemic events were observed (43.4, 49.0 mg/dl), whereof the

former was due to a human error, which resulted from having entered a too high CHO content of the corresponding meal into the algorithm. Both hypoglycemic events were immediately treated with IV glucose bolus administration (10 g each).

Deriving glucose concentrations from the combined body interface online-sensor system failed 24 times (5.4%). The online-sensed glucose concentration values exceeded the critical 20% relative difference to the reference plasma glucose concentration in 8.8% of all cases. Online sensor values differed from the reference plasma glucose concentration by -2.3 ± 14.5 mg/dl on average (relative difference -2.2 ± 13.8%).

A clinical evaluation of the monitoring part (body interface + spectroscopic online sensor) using the insulin titration error grid analysis (ITEGA)[16] revealed that 98.8% of online sensor values led to acceptable treatment, whereas 1.2% caused unacceptable violations.

Discussion and Conclusions

Study Design

Three subsystems of a closed-loop device for intensive care patients had been developed and performance tested individually in clinical trials involving healthy individuals,[4,5] T1DM patients,[6,7,11,12] and ICU patients[8,13] beforehand. In this feasibility trial, we report on the first combination of these components to form a semiautomatic closed-loop device.

Following a safe and stepwise approach, we performed this first feasibility trial in type 1 diabetic volunteers before leaping toward the target population. From a technical point of view, we neither expected the vascular body interface nor the glucose sensor to perform differently between ICU patients, T1DM patients, or healthy individuals.

The MPC algorithm was originally developed for and tested in subjects with T1DM[12] and was successfully used in the critically ill.[8,13] The present study, therefore, uses the updated controller that has already been proven to work in ICU patients. Given the arguments above and from an ethical point of view, it was thus straightforward to perform this study in T1DM volunteers instead of intensive care patients, allowing us to assess the system's safety and performance characteristics under controlled conditions using evaluation parameters, which apply to intensive care patients.

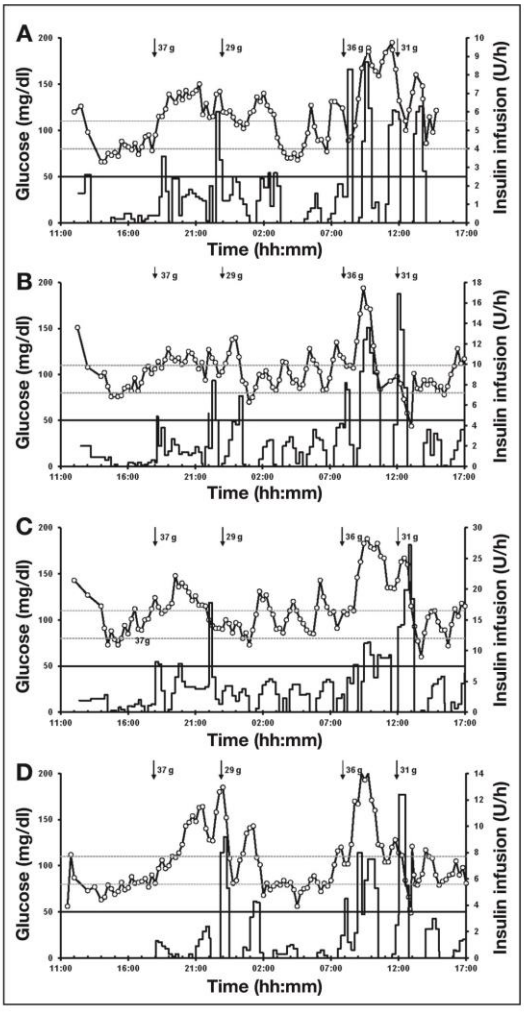

Figure 1. Glucose concentration (open circles) and corresponding insulin infusion (black solid lines) time profiles of four T1DM (A–D) in a closed-loop setup using spectroscopic glucose analysis of blood microdialysate and MPC algorithm for insulin titration suggestions. Arrows indicate food intake in gram carbohydrates. Dashed horizontal lines indicate target range for intensive care patients 80–110 mg/dl.[1] The solid horizontal line indicates the hypoglycemic threshold (50 mg/dl).

Due to the pilot character of this study and for safety reasons, we crosschecked online glucose concentration values by comparing them to reference glucose values. It would have been one option then to simply let the MPC work with whatever glucose concentration was

suggested by the online sensor. However, the results of the algorithm's performance evaluation would then have been biased by intermittent erroneous input parameters. Therefore, and again for safety reasons, we implemented a 20% relative-difference threshold for glucose concentration values for steering the algorithm.

Technical Performance Evaluation

The monitoring part of the system (i.e., the combination of vascular microdialysis and online spectroscopic glucose sensor) performed better than previously evaluated technologies[17,18] even though 8.8% of all online glucose concentration levels exceeded critical 20% relative-difference tolerance to the reference plasma glucose concentration. This is a major finding of the study from a technical point of view, which resulted from combining these subcomponents for the first time. The individual errors of the body interface and the spectroscopic online glucose sensor add up to 8.8%, which is satisfactory in our opinion but leaves space for improvement. However, only four subjects were investigated in our trial.

The ITEGA, as well as the hyperglycemic index, as useful measures of glucose control in critically ill patients suggest good glucose control, taking 110 mg/dl as the upper range of normal. Compared to other recently published closed-loop studies in T1DM patients using an SC-glucose monitoring approach,[7,17] our data suggest a better glucose control performance with respect to mean BG, mean daytime, and overnight BG, time in target range (80–110 mg/dl), and percentage of time spent in grade A and B zones of combined postprandial and outside-meal periods. Compared to closed-loop studies using BG measurement and the MPC algorithm in ICU patients,[8,14] our approach performed comparably with respect to time in target and mean BG. With respect to time in target, we report a mean overall time in target of about 47%. Comparing this result to the work of others is quite difficult as to the different natures of study designs, target ranges, etc. A study by Kovatchev and colleagues[19] in T1DM subjects reported that 78% of time spent overnight was within the target range, which was defined as 70–140 mg/dl, whereas in our presented study it is defined as 80–110 mg/dl. If we, however, apply Kovatchev's target range to our data (whole trial period), the total time in target increases to 82.8 ± 5.5%, which is a promising result for this first feasibility trial.

Safety Aspects

Two hypoglycemic events were recorded. The first (BG = 43.4 mg/dl) was caused by a human error, due to an erroneously too high nutrition entry into the MPC algorithm. The second (BG = 49.0 mg/dl) was just below the hypoglycemic threshold and also happened in the postprandial period, allowing us to conclude that the algorithm reacted rather aggressively on enteral nutrition intake. The MPC performance might be improved in that respect if it were provided additional information on the glycemic index of food to take into account the timing, peak, and duration of the glycemic surge.

Nonetheless, the results of this feasibility study are promising and we believe that our approach is worth implementing in an ICU setting, where the MPC algorithm has already proven to work efficiently.[8,13] However, before going into tests within the target population, further technical improvements have to be made and verified with respect to the controller and the monitoring part of the loop. Following our safe and stepwise approach, another series of closed-loop tests must be performed in T1DM subjects, with technical advanced subcomponents before considering first tests in ICU patients.

Another aspect of our measurement setup, which has not been illustrated in this article, is the option for monitoring further intensive care parameters in the critically ill patient, providing new tools for medical treatment.

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