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Integrated catheter system for continuous glucose measurement and simultaneous insulin infusion

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ABSTRACT

A new measurement system enables combination of continuous glucose monitoring (CGM) and insulin infusion. A sensor system comprising an optical glucose biosensor and an optical oxygen sensor is integrated into the insulin infusion catheter of an insulin pump. Both sensors rely on near infrared (NIR) phosphorescent porphyrin dyes, wherefore the signals can be read out transcutaneous and non-invasively with a custom-built phase fluorometer measurement module. The spectral properties of the indicator dyes and the optical setup of the measurement module were optimized to enable independent read-out in two channels. Dynamic ranges from 0 mmHg to 160 mmHg oxygen and 0 mg/dL to 360 mg/dL glucose (LOD 2 mg/dL) are covered by the oxygen and the glucose sensor, respectively. *In-vivo* measurements in pigs demonstrate good correlation of reference blood glucose levels and glucose values obtained with the presented sensor system. The evaluation of the clinical accuracy of the system with Clarke Error Grid Analysis showed similar results to CGM-devices currently on the market.

1. Introduction

Diabetes mellitus is a group of complex metabolic diseases resulting from a total or partial lack of insulin. According to the World Health Organization (WHO) approximately 347 million people worldwide suffer from diabetes (WHO, 2013). Continuous glucose measurement and adapted insulin delivery are necessary to achieve good glycemic control, normalize the diabetic's glucose metabolism and decrease the danger of acute and long-term complications of type 1 diabetes (T1D). Currently diabetics self-monitor their blood glucose level by obtaining a finger-prick sample of capillary blood, which they apply to a test strip and portable meter for analysis. This painful procedure is carried out several times a day and may result in cornification and diminished sensibility of the fingers. Moreover, episodes of hypo- or hyperglycemia that occur at night or between sampling can be missed. It has been shown that CGM is highly beneficial for children with T1D (Lagarde et al., 2006) but also for adults with T1D and nocturnal hypoglycemic events (DeVries et al., 2004; Weinzimer et al., 2008; Wentholt et al., 2007). CGM is not achievable with the currently popular therapy, as diabetic patients do not tolerate a more frequent measurement. The research of last decades was aimed at the development of a closed loop system and an artificial pancreas with automated continuous glucose measurement and adjusted insulin delivery. The MiniMed Paradigm® REAL-Time Revel™ System (Medtronic, 2014a) or the MiniMed® 530G with Enlite® (Medtronic, 2014b) are examples for commercially available insulin pumps with integrated CGM-system. Other CGM-systems on the market are the Seven®Plus (Dexcom, 2012), the FreeStyleNavigator II (Abbott Diabetes Care, 2013) or the GlucoDay®S (A. Menarini Diagnostics, 2014).

Compared to conventional (*e.g.* amperometric) glucose sensors, optical sensors benefit from the absence of electromagnetic interferences, simple design and handling as well as low cost. Miniaturization of optical sensors is easily achievable without compromising performance and dramatically reduces repulsion reactions. Evidently, optical sensing technology can provide an interesting alternative to more established electrochemical sensors and will contribute to better flexibility concerning the design of CGM-systems, their cost, materials used, *etc.*

Several schemes for optical glucose monitoring have been reported (Pickup et al., 2005; Borisov and Wolfbeis, 2008; Steiner et al., 2011). Examples are affinity sensors based on natural receptors like concanavalin A (Ballerstadt and Schultz, 2000;

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Cummins et al., 2013) or synthetic receptors like boronic acids (Mader and Wolfbeis, 2008), as well as fluorescent hydrogels (Heo et al., 2011) or enzymatic biosensors which rely on glucose oxidase (GOx). In contrast to affinity sensors, enzymatic glucose sensors are distinguished by a very high selectivity (Pasic et al., 2007) and are virtually "blind" to other saccharides. Oxygen sensors which act as transducer in enzymatic glucose sensors are quite established (Amao, 2003; Quaranta et al., 2012) and can be read out with phase fluorometry using compact and potentially low cost devices. Adversely, tissue oxygenation affects the response of this type of sensor, making additional measurement of oxygen partial pressure (pO_2) essential (Li and Walt, 1995). This results in higher complexity of the composite glucose sensor. In order to ensure accurate compensation for oxygen fluctuations, both sensor elements (glucose and reference oxygen sensor) should be located in close proximity. Although the proof-of-concept was previously demonstrated for the combination of two fiber-optic sensors (Pasic et al., 2007), the array was rather bulky and did not include an insulin catheter. Evidently, integration of the insulin catheter and the sensor into one element allows for a significantly patient-friendlier and more compact setup. This is a challenging task since the optical glucose and oxygen sensor should be read out through the skin. The state of the art UV–vis probes are poorly suitable for subcutaneous measurements and can be applied only for proof-of-concept studies of such "Smart Tattoos" (Ballerstadt and Schultz, 2000; McShane et al., 2000). Recently much progress was achieved in the area of phosphorescent oxygen probes which absorb and emit in the so-called NIR optical window (Weissleder, 2001). This spectral region is distinguished by lower light absorption by the skin pigments, hemoglobin and water and by weaker auto-fluorescence from the tissue (Thompson, 1994).

The long-term performance of implantable sensors is usually compromised by accumulation of metabolically active cells. They accumulate on the surface of the sensor and consume glucose and oxygen at accelerated rate (Frost and Meyerhoff, 2006). Additionally, the formation of a fibrous capsule around the sensor alters glucose mass transfer. This can be solved by taking a blood sample and recalibrating the sensor after local environment has stabilized. Furthermore, the use of inexpensive optical sensors combined with insulin catheters makes it possible to overcome the complications mentioned above by replacing the sensor every 2–3 days.

In this contribution we present a system, which allows for continuous glucose measurement and simultaneous insulin infusion. An optical sensor system is integrated into the insulin infusion catheter connected to the insulin pump and requires only one injection site. Like most CGM-systems, the sensor measures glucose in the interstitial fluid (ISF), which is at equilibrium with blood glucose levels (Simonsen et al., 1994). Frequent measurements deliver more information and help to identify hypo- or hyperglycemic events, which might stay undetected with the finger-prick method. In future work the system will be expanded to an artificial pancreas with help of an algorithm controlling the insulin pump in dependence of the measured glucose levels. The almost pain-free solution is expected to greatly simplify glucose measurement for the patients and to decrease the risk of acute and long-term complications conditional on diabetes.

2. Material and methods

2.1. Materials

Glucose oxidase (EC 1.1.3.2 from *Aspergillus niger*, 200 U/mg), 4-tert-butylstyrene, aluminum oxide type CG-20, sodium dodecyl sulfate and 2,2′-azobis(2-methylpropionitrile), divinyl-benzene, glutaraldehyde (25% aqueous solution), poly(styrene) (average Mw 250,000) and ethylcelullose (48.0–49.5% ethoxyl basis) were purchased from Sigma-Aldrich (www.sigmaaldrich.com). Albumin fraction V from bovine serum, D-(+)-glucose and all solvents were obtained from Roth (www.carlroth.at), nitrogen and synthetic air (all of 99.999% purity) from Air Liquide (www.airliquide.at) and hydrogel HydroMed D4 (AdvanSource Biomaterials, 2010) from AdvanSource (www.advbiomaterials.com). Deionized water was used for the preparation of all aqueous solutions.

The luminescent dye platinum(II)-*meso*-tetra(4-fluorophenyl) tetrabenzoporphyrin was synthesized according to Borisov et al. (2009). The synthesis of platinum(II)-6-aza-13,20,27-triphenyltetra(tert-butylbenzo)-porphyrin is described in Supporting information. The 20% glucose solution Glucosteril® and a 0.9% physiological NaCl-solution for *in-vivo* tests were purchased from Fresenius Kabi (www.fresenius-kabi.de) and the insulin Novorapid 100 U/mL from Novo Nordisk (www.novonordisk.com).

Polytetrafluoroethylene-tubes were purchased from Bola (www.bola.de) and poly(ethylene glycol terephthalate)-foil (Mylar®) from Goodfellow (www.goodfellow.com). Intravenous catheters Tro-Venocath 3+ were obtained from Troge Medical (www.trogemedical.de).

2.2. Preparation of poly-tert-butylstyrene-particles (ptBS)

Prior to the polymerization, 4-tert-butylstyrene (tBS) and divinylbenzene (DVB) were cleaned on an aluminum oxide column to remove the inhibitor. 10.9 mL of tBS and 2.8 mL of DVB were mixed and added to 400 mL of a 0.25% (w/v) aqueous solution of sodium dodecyl sulfate (SDS). The mixture was homogenized for 2 min with a homogenizer (Miccra D1, Gerber Instruments, Switzerland) and for 5 min in an ultrasonic bath (Transsonic Digital S, Elma, Germany). The resulting emulsion was transferred into a 1 L-two-necked round-bottom flask and stirred under strong nitrogen flow for 40 min. 200 mg of 2,2′-azobis(2-methylpropionitrile) were added and the emulsion was warmed to 95 °C. After approximately 7 h, the reaction mixture was cooled to room temperature and transferred into a beaker. 600 mL of acetone were added under stirring to precipitate the resulted cross-linked polymer particles. The dispersion was centrifuged at 4000 rpm for 5 min (Rotofix 32, Hettich, Germany). For cleaning, the precipitate was suspended in approximately 60 mL of a solvent, ultrasonicated for 10 min and centrifuged again. This cleaning procedure was carried out three times with acetone, twice with a mixture of acetone, ethanol and water (2:1:1 v/v) and twice with ethanol. The particles were dried overnight in a drying oven (ED115, Binder, Germany) at 60 °C and the product (ptBS) was then homogenized in a mortar.

2.3. Staining of ptBS-particles

A solution of 5 mg platinum(II)-*meso*-tetra(4-fluorophenyl)tetrabenzoporphyrin (PtTPTBPF) in 6 mL chloroform was slowly added to a 10% (w/v) dispersion of ptBS in $CHCl_3$. The mixture was stirred for 15 min and ultrasonicated for another 15 min. The solvent was removed by evaporation under nitrogen flow. The stained PtTPTBPF–ptBS-particles were dried overnight at 60 °C and then homogenized in a mortar.

2.4. Preparation of the glucose biosensor

The surface of the polytetrafluoroethylene (PTFE)-tube simulating the insulin infusion catheter was activated shortly before the coating procedure by oxygen plasma etching (FEMTO UHP, Diener Electronic, Germany). The process was carried out at a basic pressure of 0.3 mbar and a plasma power of 100 W and 40 kHz for 60 s.