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A label-free fiber-optic Turbidity Affinity Sensor (TAS) for continuous glucose monitoring

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ABSTRACT

In this paper, we describe the concept of a novel implantable fiber-optic Turbidity Affinity Sensor (TAS) and report on the findings of its in-vitro performance for continuous glucose monitoring. The sensing mechanism of the TAS is based on glucose-specific changes in light scattering (turbidity) of a hydrogel suspension consisting of small particles made of crosslinked dextran (Sephadex G100), and a glucose- and mannose-specific binding protein – Concanavalin A (ConA). The binding of ConA to Sephadex particles results in a significant turbidity increase that is much greater than the turbidity contribution by the individual components. The turbidity of the TAS was measured by determining the intensity of light passing through the suspension enclosed within a small semi-permeable hollow fiber (OD: 220 μm, membrane thickness: 20 μm, molecular weight cut-off: 10 kDa) using fiber optics. The intensity of measured light of the TAS was proportional to the glucose concentration over the concentration range from 50 mg/dL to 400 mg/dL in PBS and whole blood at 37 °C ($R > 0.96$). The response time was approximately 4 min. The stability of the glucose response of the TAS decreased only slightly (by 20%) over an 8-day study period at 37 °C. In conclusion, this study demonstrated proof-of-concept of the TAS for interstitial glucose monitoring. Due to the large signal amplitude of the turbidity change, and the lack of need for wavelength-specific emission and excitation filters, a very small, robust and compact TAS device with an extremely short optical pathlength could be feasibly designed and implemented for in-vivo glucose monitoring in people with diabetes.

1. Introduction

The International Diabetes Federation (IDF) has predicted that the prevalence of diabetes will further increase from 6% (246 million people) in 2007 to 7.3% (380 million) in 2025 worldwide. The fastest increase in diabetes prevalence is expected to occur in developing countries. Type 1 diabetes is expected to rise at an alarming rate of 3% each year (Shaw et al., 2010). External restoration of glycemic levels through diet, lifestyle, and insulin therapy have been shown to dramatically reduce associated comorbidities (The Diabetes Control and Complication Trial Research Group, 1993). Tremendous progress has been made with regard to the development of glucose-monitoring technologies, and control algorithms for insulin infusion ("Artificial Pancreas") (Hovorka, 2006, Place et al., 2013, Russell et al., 2012). The current crop of commercially available Continuous Glucose Monitoring Sensors (CGMS – Seven by Dexcom, Paradigm by Medtronic-Minimed) are based on electro-enzymatic detection technology. They require several recalibrations per day to avoid sensor drift, and can be prone to certain electrode-sensitive compounds (e.g. acetaminophen). In order to overcome these issues, novel glucose-sensing technologies are warranted, which may provide a superior robustness, thus safety, while maintaining high accuracy levels during in-vivo operation. This is especially of utmost importance when integrated within a closed-loop system. Various optical affinity-based glucose sensing technologies have been successfully demonstrated as potential alternatives for glucose-monitoring in people with diabetes (Schultz et al., 1982, Ballerstadt et al., 2012, Colvin and Jiang, 2012, Meadows and Schultz, 1993, Romey et al., 2012, Tolosa et al., 1997, Worsley et al., 2007). The shortcomings of most fluorescence-based sensing technologies are potential signal degradation due to gradual destruction of the fluorescent dyes via photobleaching (Ballerstadt et al., 2004), or chemical attack from Reactive Oxygen Species (ROS) (Colvin and Jiang, 2012). To overcome the weakness of the fluorescence sensor approach, our group has demonstrated proof-of-concept of a label-free optical glucose sensor (Ballerstadt et al., 2007a,b). The mechanism was based on measuring glucose-sensitive changes in light scattering (turbidity) with optical coherence tomography (OCT) (Ballerstadt

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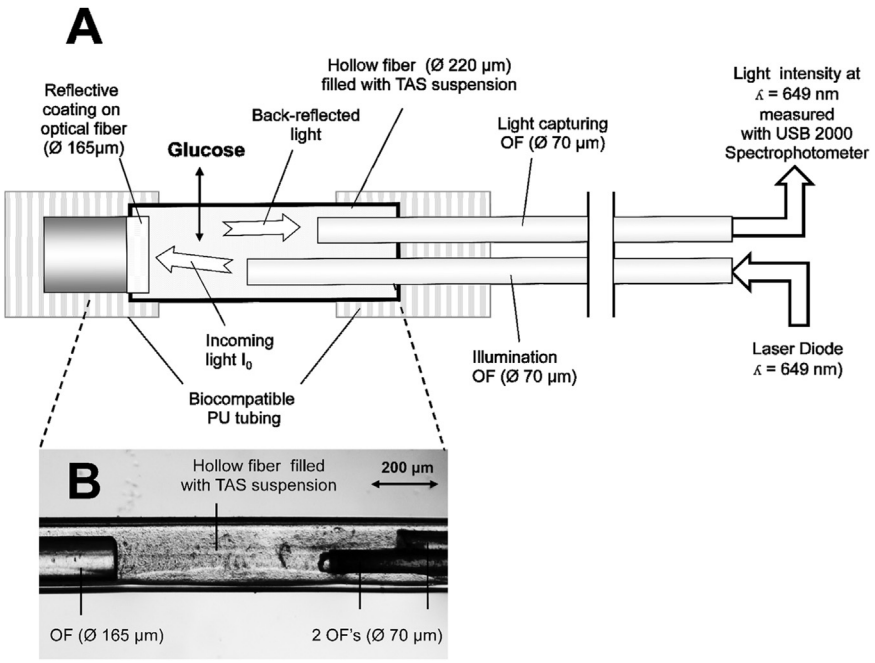

Fig. 1. *Illustration (A) and Photographic Image (B) of the TAS.* See text for further explanation.

et al., 2007a,b). The chemistry was composed of small crosslinked dextran-particles (Sephadex) and the glucose-specific protein Concanavalin A (ConA). The binding of ConA to the hydrogel particles resulted in a light scattering coefficient that was 8 times higher than the sum of the scattering coefficients of the individual components. The addition of glucose led to the reversible decrease in turbidity. Despite the promising data, the complexity of OCT operation to facilitate turbidity measurements through skin can become extremely challenging, because of strong signal attenuation by skin and due to the difficulty to reproducibly implant the turbidity sensor at less than 500–300 µm into subcutaneous tissue. Therfore, a practical solution may still take years. Therefore, in this paper the objective of the study was to simplify the optical interrogation technique by measuring the intensity of light passing through the glucose-sensitive suspension within a small – potentially implantable – semipermeable hollow fiber. At low glucose levels the intensity of light passing through the hydrogel is low due to high light scattering. However, in the presence of high glucose levels, the intensity of light passing through the suspension is high due to low light scattering. The central element of the TAS is a semipermeable hollow fiber containing the ConA/Sephadex hydrogel suspension (see Fig. 1A). The hollow fiber has an outer diameter of 220 µm and a membrane thickness of approximately 20 µm. The molecular weight cutoff is approximately 10 kDa. It allows glucose to diffuse in and out of the lumen of the hollow fiber, but prevents ConA and the hydrogel beads from leaking out. To enable light measurements in a transmission mode, two 70-µm optical-fibers are located inside the hollow fiber – one fiber for coupling incident light into the lumen using a laser diode (λ=649 nm), and the other to couple back the light passing through the TAS suspension when returning from the reflection against the mirror-like surface at the distal end of the hollow fiber. Both optical fibers were positioned apart in order to minimize the background signal by optical "bleeding". A microscopy photographic image of a mock sensor is shown in Fig. 1B. The experiments in this study were designed to establish proof-of-concept data of the TAS by assessing the in-vitro performance of the sensor device under physiological conditions for its potential use as an implantable glucose sensor for people with diabetes.

2. Material and methods

2.1. Chemicals

Concanavalin A (ConA, Type VI, Sigma-Aldrich, St.Louis, MO), Sephadex G100 (fine 20–80 µm, dry), dextrose and phosphate buffered saline (PBS) were purchased from Sigma Aldrich (USA).

2.2. Shredding of Sephadex hydrogel particles

We determined in previous studies (Ballerstadt et al., 2007a,b) that wet hydrogel particles with bead sizes ranging from 1 to 30 µm were optimal for measuring high glucose-dependent light scattering changes of the suspension. To achieve this, Sehadex G100 (dry diameter 20–80 µm) was swollen in distilled water for 2 h. Then 600 µl of the swollen suspension was transferred to 1.5-ml Eppendorf tubes, and 0.19 mg of ZrO*x* beads with an average size 0.15 mm and 0.19 mg with a size of 2 mm were added to the suspension. The tubes were then placed in a bullet blender (Next Advance Inc., Averill Park, NY, USA), and shredded at 4 °C for a total of 20 min. After completion, the shredded Sephadex G100 hydrogel particle were separated from the ZrO*x* beads by gravity and removed from the tube. Shredded hydrogel material was then stored at 4 °C until use.

2.3. Preparation of sensor components

A solution containing ConA was slowly added to a suspension of shredded G100 hydrogel beads to a final ConA-concentration of 20 mg ml^{-1}. To fill short cellulose-based hollow fiber segments (Membrane GmbH, Wuppertal, Germany) at a length of approximately 5 cm with this suspension, dextrose at a concentration of approximately 500 mg/dL was added to the suspension to lower its viscosity for easier filling. The suspension was aspirated with an automatic pipette (tip volume 1–200 µl), and tightly pressed into a 1–10 µl tip which had a short hollow fiber segment adhered by glued to its distal end. By slowly turning the plunger screw on the pipette the hollow fiber was filled with the TAS suspension by visual observation. Then sections filled with the suspension that were free of air-bubbles were cut and sealed with loctite 4013 glue