APPLICATION OF AN ENZYMATIC MICROREACTOR COUPLED WITH MICRODIALYSIS FOR CONTINUOUS MONITORING OF SUBCUTANEOUS GLUCOSE IN RATS

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ABSTRACT

We present here an application of a newly-designed enzymatic microreactor (EMR) for continuous glucose monitoring in rats. The EMR was based on electrochemical detection and was coupled with a microdialysis probe which was inserted into the subcutaneous tissue of anesthetized rats. The performance of the EMR was evaluated by modulating blood glucose concentrations with intravenous injections of glucose or insulin, with saline as a control. Blood glucose (BG) was frequently measured as a reference value for comparison with the EMR. The difference between glucose values produced by BG sampling and EMR monitoring are also discussed.

KEYWORDS: Enzymatic microreactor, Continuous glucose monitoring, Microdialysis, *In vivo*

INTRODUCTION

Continuous glucose monitoring allows metabolic status to be assessed, and the fluctuation of glucose concentrations to be controlled over longer periods of time. To date, the Subcutaneous Continuous Glucose Monitoring system (SCGM, Roche Diagnostics GmbH) has proven to be a good candidate for monitoring glucose in patients with diabetes, and has been used in clinical studies. This system provides safe and reliable continuous glucose monitoring over longer periods (days) [1]. However, the overall system configuration is rather bulky, and the system exhibits a long lag time between sampling and analysis result (over 30 min). Therefore, the concept of a miniaturized SCGM system using small volumes of reagents to carry out the solution-based reaction of glucose oxidase (GOx) with glucose constitutes a promising approach.

Chip-based microfluidic technologies provide an excellent alternative for conventional glucose monitoring approaches such as the SCGM. Advantages of these technologies include dramatically reduced consumption of chemical reagents, cost effectiveness and reaction time. Micrometer-sized fluidic channels enable the reaction of GOx and glucose on the nL scale. These nL volumes are also compatible with microdialysis sampling and its applications. Using lower flow rates for microdialysis sampling, it is possible to achieve a high recovery of glucose from the subcutaneous tissue. Additionally, microdialysis is a safe technique; only Ringer's solution is perfused through the lumen of the probe, which is separated from the body by a semi-permeable membrane. There are no known adverse side effects. Since microdialysis is used to collect samples from the interstitial fluid (ISF), the microfluidic device also does not impose any additional biocompatibility problems or disturb tissue.

The aim of this study is to evaluate an EMR coupled with microdialysis for continuous glucose monitoring in the subcutaneous tissue of rats. We present preliminary results to illustrate the potential of the system for *in vivo* applications.

EXPERIMENTAL

The relative glucose recovery for microdialysis probes was determined using an *in vitro* approach and a colorimetric method to analyze for glucose. The membrane of the microdialysis probe was polyacrylonitrile, with a 45-50 kDa molecular weight cut-off; probe membranes 1 or 3 cm in length were tested. The recovery is defined as Recovery = C_{out} / C_{beaker}, with C_{out} the concentration of glucose in the dialysate and C_{beaker} the initial concentration of the glucose in the medium being sampled.

An EMR based on electrochemical detection has been developed, as presented previously by our group [2]. This device has a 10-cm-long reaction channel with a groove array for chaotic mixing [3]. The thin-film Pt electrode was coated with overoxidized polypyrrole to prevent protein adsorption and reaction with interfering substances. Figure 1 shows a schematic diagram of the experimental set-up for *in vitro* calibration of a microdialysis probe coupled to a chip. A microdialysis probe was immersed in glucose-containing solution and perfused with Ringer's solution at a flow rate of 0.5 μL/min. A 150 U/mL GOx solution was introduced into the other inlet of the EMR at a flow rate of 1.5 μL/min. The temperature was maintained at 37 ºC. The *in vitro* calibration curve was prepared by injecting varying volumes of 1 M stock glucose solution into the beaker in which the probe was immersed, to achieve different concentrations of glucose (2.1 mM, 5.6 mM, 10.1 mM, 15.0 and 20.6 mM). The H_2O_2 produced by the reaction between glucose and glucose oxidase was oxidized at the integrated microelectrodes at an applied potential of 700 mV with respect to Ag/AgCl. The resulting current was recorded as a function of glucose concentration.

The preliminary *in vivo* experiments were performed in the subcutaneous tissue of anesthetized rats (male Wistar rats (Harlan, The Netherlands)), and EMR performance was evaluated in response to intravenous injections of saline, 20 % (w/v) glucose and 5 U/kg insulin, delivered via a catheter in the jugular vein. The microdialysis probe (with 1-cm-long exposed membrane) was inserted into the subcutaneous tissue in the dorsal region of the rat. Blood glucose (BG) was determined with an Accu-Chek analyzer using colorimetric test strips (Roche Diagnostics GmbH, Germany).

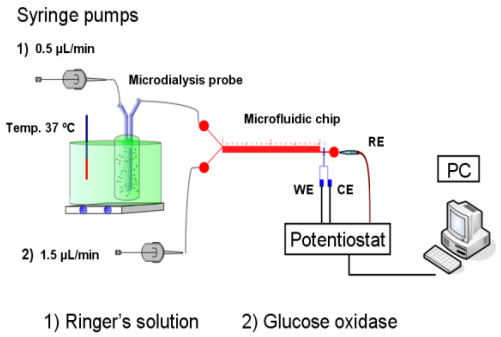

Figure 1: In vitro *experimental set-up.*

RESULTS AND DISCUSSION

Results of the *in vitro* characterization of probe glucose recovery show that as the flow rate was increased, recovery was decreased, due to reduced times for diffusion of glucose over the membrane. The recovery for the 1-cm probe was determined to be approximately 95% at a flow rate of 0.5 µL/min (Figure 2). Prior to its use *in vivo*, the EMR was also calibrated *in vitro* in medium with different glucose concentrations. The sensitivity and correlation coefficient R were 4.5 nA/mM and 0.9895, respectively, as shown in Figure 3.

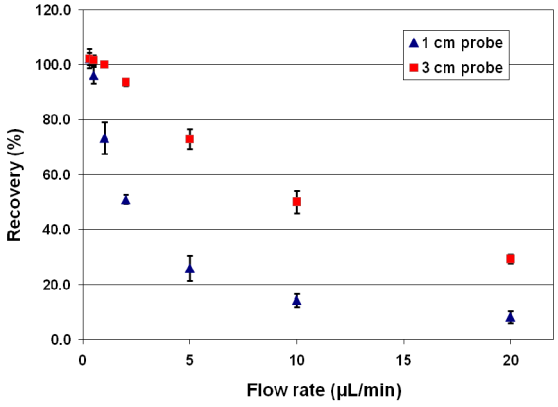

Figure 2: In vitro *recovery of glucose at flow rates between 0.3 and 20 µL/min (n=3).*

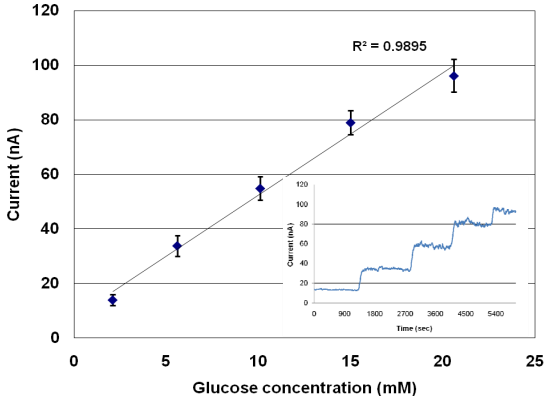

Figure 3: In vitro *calibration curve (n=3). Data from series of measurements is shown in the inset.*

Figure 4 shows subcutaneous glucose concentrations over time, expressed as percentage of baseline, with arrows indicating respective administrations. An increase of about 20 % was observed when glucose was administered, while an 80 % decrease resulted upon administration of insulin. The glucose concentrations measured by BG sampling and the EMR are compared in Figure 5. Blood glucose was measured by withdrawing a blood sample every 15 min. The EMR signal followed the changes in BG level, but is consistently lower. This difference is mainly due to the inherently different glucose levels in blood and ISF in subcutaneous tissue; ISF levels are also expected to be lower because glucose must diffuse into the ISF from the blood. Along that path, some glucose is taken up by cells surrounding the EMR. Implantation of the microdialysis probe also changes the physiological conditions somewhat, because the tissue is damaged during probe insertion and inevitably affects glucose monitoring for several days before tissue has recovered [4].

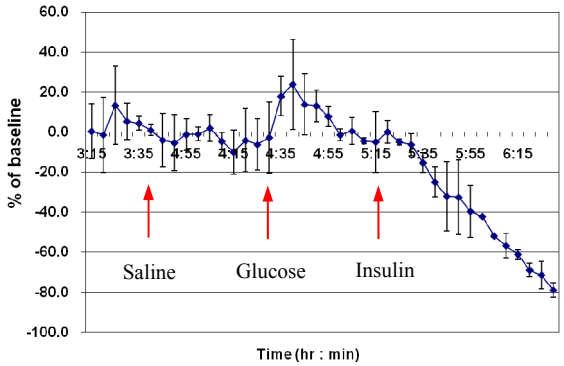

Figure 4. In vivo preliminary result in anesthetized rats (n=2).

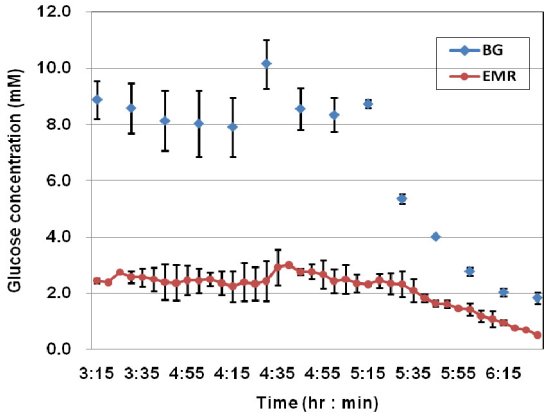

Figure 5. In vivo comparison with BG sampling and EMR signal (n =2).

CONCLUSION

We have successfully demonstrated an *in vivo* application of an EMR coupled with microdialysis. These preliminary results illustrate a possible application of microfluidic chips for continuous monitoring of subcutaneous glucose in rats. As a proof of principle, it was possible using the microSCGM system to register changes in subcutaneous glucose that were caused by experimental modulation of glucose with intravenous administrations of glucose or insulin.

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