MICRO-FLOW-SYSTEMS WITH BIOSENSOR DETECTION FOR IN-VIVO USE

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ABSTRACT

In order to overcome the limitations of commercially available microdialysis systems we used planar micro-technologies to realise a miniaturized device comprising connection fluidics integrated to a biosensor array flow cell. In this way overcoming the need for and the risks of the assembly and also minimising the internal volume.

The microdevice consists of a dialysis sampling system integrated to the flow-through cell of a microfabricated biosensor array for the simultaneous on-line monitoring of glucose and lactate.

The fluidic connections between the different device's components are realized by subsequent processing of stacked dry resist layers on a plastic support that provides also the means for electric connections. The performance of the device was evaluated in vitro. Recoveries of over 95% for both analytes were achieved when flow rates of the perfusion fluid ≤ 0.5 µl/min were used. At this flow rate, the response time of the device was 2.4 min, which is acceptable for on-line analysis. The device showed a long-term run stability both in buffer and serum samples.

Keywords: microdialysis, microfabrication, monitoring.

INTRODUCTION

Microdialysis is an in vitro technique to investigate extra cellular liquids in various tissues. It is based on a molecular diffusion process across a semipermeable membrane. In practical applications sterilized hollow fibers of a dialysis membrane are implanted and, by tubing connections to a precision pump, perfused at a low flow rate with an isotonic fluid. The dialysate is then transferred to a flow-through cell outside the body equipped with an appropriate sensor for the analyte.

The gradient of the analyte concentration across the membrane is the driving force of the diffusion process. Although micro-dialysis became a well established routine tool for the minimally invasive probing of metabolism, on line monitoring in combination with biosensors is still in the research state. There are many benefits in using microdialysis with subsequent biosensor detection. The biocompatibility issue is moderated as compared to in vivo sensors and the release of active agents to the monitoring site becomes possible.

These benefits have drawn broad attention to the microdialysis field. Although considerable progress has been made, the applications suffer mainly from the high priced microdialysis probes, the manual microdialysis-sensor coupling procedure, and the

monitoring delay due to the internal volume between sampling membrane and sensors. Additional delay time is needed for pooling the dialysate to get enough volume for the analysis. The time delay hinders to intervene during the measurement, the pooling gives only an average concentration over the observed interval. The integration of a miniaturized dialysis probe with a microsystem with biosensors enabled the device to measure simultaneously and continuously glucose and lactate with reduced lag times.

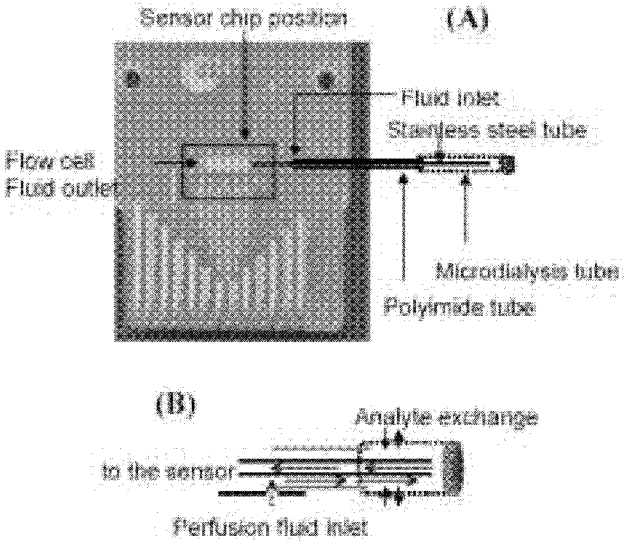

Fig.1 Picture of the PCB used for device fabrication (A) and schematic drawing of the sampling probe (B).

EXPERIMENTAL

The device is equipped with a microdialysis probe integrated on the flow through channel of a microfabricated electrochemical sensor. The aim of this work was to integrate a dialysis probe on a microfabricated sensor device in order to obtain a complete microsystem suitable for continuous monitoring of glucose and lactate. For this purpose a specific dialysis probe was designed and a PCB was appropriately converted to support both the sensor chip and the dialysis probe.

The biosensor array consists of up to five amperometric enzyme membrane sensors, a temperature sensor, and one silver/silver chloride reference electrode. The immobilization of the biocomponents by nanodispensing and subsequent UV exposure. The respective oxidases like glucose oxidase and lactate oxidase were immobilized onto the working electrodes in a photo crosslinked pHEMA membrane and additionally covered by a diffusion limiting and a catalase containing top layer.

A polyacrilonitrile fiber contained in an artificial kidney was used as dialysis membrane. The membrane cut-off value was 50 kDa. The perfusion fluid was pumped to the probe through a polyimide tube placed above the fluid inlet. In the dialysis probe the perfusion fluid exchanged substances with the surrounding medium and then the dialysate was driven by a stainless steel tube to the sensor flow channel where the fluid outlet hole was situated (Fig. 1). The insertion of the polyimide tube between the fluid inlet and the

dialysis probe provided the ability to vary the length of the whole probe without changing the dialysis active surface. The polyacylonitrile fiber is the part of the device that will be introduced in the human body and it was selected for biocompatibility reasons.

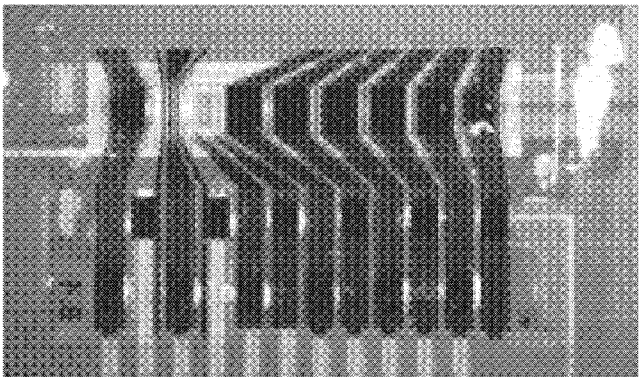

Fig.2 Micrograph of the assembled biosensor array chip. On the right side the microdialysate enters the 0.36 μl flow cell.

A printed circuit board (PCB) that comprised conductive pads, a plug for the potentiostat and a gold counter electrode was used for the assembly of the biosensor device. The flow cell, as well as, a 4 mm long 0.2 mm wide channel extending from the left end of the flow channel to the fluid inlet hole were created on the PCB using a dry film resist insulation layer. Another wider channel (approximately 0.5 mm) was milled onto the PCB from the fluid inlet hole to the PCB edge. Finally, the holes for fluid inlet and outlet were drilled on the PCB. A 4 cm long stainless steel tube was inserted through the 0.2 mm wide channel to the flow cell and fixed on the board with adhesive. Subsequently, the sensor chip was assembled using a conductive adhesive and the flow channel was sealed also with adhesive. The volume of the flow channel created by this way was 0.36 μl (5.5 x/ 0.7x 0.094 mm^3). A polyimide tube of about 2.5 cm long was then placed around the stainless steel tube and driven through the 0.5 mm wide channel to the fluid inlet hole. The polyimide tube was fixed in that position and the channel was sealed by an appropriately cut glass chip bonded with adhesive. The final step for the preparation of the device was the assembly of the microdialysis tube. The tube was placed around the stainless steel tube up to the end of the polyimide one and was fixed there with adhesive. The other end of the tube was closed with adhesive so that the distance between the end of the stainless steel and the micro-dialysis tube was about 0.5 mm. The total length of the active microdialysis probe was 10 mm. The miniaturized device was used in vitro. High sampling efficiency of the microdialysis probe was achieved by appropriate selection of the perfusion fluid flow rate. Response times varying from 1.5 to 3.0 min were determined for flow rates ranging between 1 and 0.2 μl/min (see Fig. 3). Interference from electroactive substances was almost negligible. The device showed excellent stability under continuous operation for at least 5 days and sensitivity variation less than 3% over a period of 15 days.

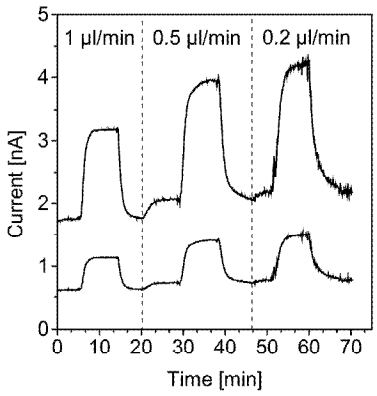

Fig.3 Effect of the perfusion fluid flow rate onto signal transition. The concentrations of glucose and lactate in the sample were changed from 5 and 2.5 mM to 10 and 5 mM respectively and vice versa.

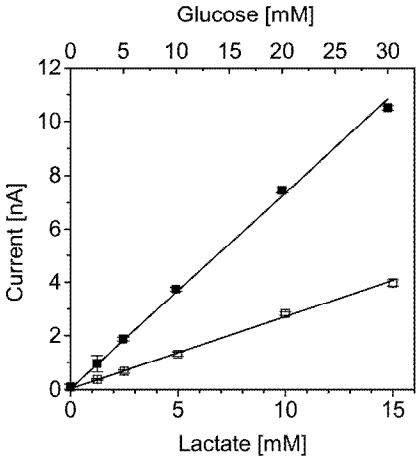

Fig.4 Calibration curves for glucose (upper curve) and lactate (lower curve) obtained with the device developed using a flow rate of 0.5 µl/min. Each point was measured in triplicate.

The linear response range was up to 30 mM glucose and up to 15 mM for lactate (see Fig. 4) with sensitivities of 0.36 nA/mM glucose and 0.27 nA/mM lactate. Calibration curve intercept is below 0.1 mM for each analyte.

REFERENCES

I. Moser, G. Jobst, G.A. Urban, "Biosensor Arrays for Simultaneous Measurement of Glucose, Lactate, Glutamate, and Glutamine", Biosensors & Bioelectronics, **17/4**, 297-302, 2002.