AN ENZYMATIC MICROREACTOR FOR CONTINUOUS GLUCOSE MONITORING

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ABSTRACT

We demonstrate a new application for chaotic mixing, namely the fast reaction of glucose oxidase (GOD) and glucose in solution. Two different types of chaotic mixing channels with arrays of either slanted or herringbone grooves were fabricated in poly(dimethylsiloxane) (PDMS) and compared to channels containing no grooves. A thin-film Pt electrode was positioned at the end of the fluidic channel as an on-chip electrochemical detector of the reaction product, H2O2. The calibration curve was linear for glucose concentrations of 0 to 10 mM, with a sensitivity of 80 mA/M cm² in the clinical range of interest.

KEYWORDS: Enzymatic microreactor, Continuous glucose monitoring, Chaotic mixer, Electrochemical detection

INTRODUCTION

The long-term goal of this project is to realize an autonomous, portable sensing system for continuous in vivo glucose monitoring, based on the reaction in solution of glucose oxidase (GOD) with glucose to produce H2O2.

A continuous in vivo glucose monitoring system should provide clinically relevant information in real time over longer periods (days) for better control of glucose levels [1]. To date, systems have commonly relied on electrochemical detection (ECD), with GOD immobilized directly onto the electrode to minimize enzyme consumption and facilitate detection of the H2O2 produced. However, immobilization of enzyme layers which are robust enough to perform over many days continues to be difficult. Enzyme activity diminishes over time, and sensing layers are prone to fouling. Additionally, monitoring systems are still bulky and uncomfortable to wear. We have adopted an alternative microfluidic approach, in which nL amounts of sample and enzyme are freely and rapidly reacted in a microreactor comprising a channel for chaotic mixing, as first presented by...
Stroock et al. [2]. Integrated Pt electrodes (uncoated) detect the H$_2$O$_2$ produced. The use of microfluidics has several advantages, including fast response times (fast reactions), flowrates which are compatible with microdialysis sampling, economic use of enzyme and possibilities for overall system miniaturization. ECD instrumentation can also be made small and portable.

**EXPERIMENTAL**

The microchannels were replicated in poly(dimethylsiloxane) (PDMS), and sealed with a glass wafer with thin-layer Pt electrodes on the surface (Figure 1). A positive photoresist (PR) 4562 mold was made on a silicon wafer using two steps of standard photolithography to make a chaotic mixing channel with an array of grooves [2]. Metal electrodes (Ti/Pt) were deposited by a lift-off process on a Borofloat glass wafer. The PDMS slab and glass with electrodes were irreversibly bonded after UV-ozone treatment to oxidize the bonding surfaces. Figure 2 shows a fabricated device with a 200-µm-wide, 35-µm-deep channel. The groove structures in the mixing channel were 6 to 8 µm deep. For characterization of mixing, fluorescence detection was applied to determine fluorescence quenching of fluorescein with KI. At a flow rate of 1 µL/min, the intensity distribution across the entire channel had a standard deviation of less than 0.1 at a distance of 1 cm from the Y-junction, indicating that mixing was almost complete at this point.

Glucose measurements were carried out using a potentiostat (CH Instruments) under continuous-flow conditions (Figure 3). Two Pt electrodes were used as working and counter electrodes, respectively. The Pt electrodes were located 20 mm downstream from the Y-junction. A Ag/AgCl miniaturized reference electrode was positioned in the reservoir at the end of the device.

**RESULTS AND DISCUSSION**

Figure 4 shows three different types of PDMS microfluidic channel with integrated electrodes, containing no grooves, slanted grooves, or herringbone grooves. Cyclic voltammetry was carried out in 1mM Fe(CN)$_6^{3-}$/Fe(CN)$_6^{4-}$ prepared in 20 mM phosphate-buffered solution (pH = 7.2) containing 0.1 M potassium nitrate as a supporting electrolyte at a flow rate of 1 µL/min and a scan rate of 20 mV/s. Figure 5 shows higher oxidation and reduction currents for channels with slanted or herringbone grooves than for unstructured channels. This is probably caused by higher
local flowrates over the electrode surfaces in the chaotic mixers, leading to thinner diffusion layers at these surfaces and thus improved analyte delivery and higher currents. Figure 6 shows the amperometric response to different glucose concentrations when a 300 U/mL GOD solution was mixed with sample in a channel with slanted grooves at a flow rate of 1 μL/min. The curve calibration curve was linear for glucose concentrations of 0 to 10 mM, with a sensitivity of 80 mA/M cm².

![Figure 4](image1.png)

(a) (b) (c)

Figure 4. Photographs of different types of PDMS channels with integrated electrodes: a) no grooves, b) slanted grooves, c) herringbone grooves.

![Figure 5](image2.png)

Figure 5. Comparison of cyclic voltammograms for the three different types of microfluidic channel.

![Figure 6](image3.png)

Figure 6. Glucose measurement with various concentrations of glucose.

CONCLUSIONS

We have successfully introduced an enzymatic glucose reactor based on chaotic mixing into a microfluidic channel network for continuous glucose monitoring. This system will be further developed and coupled with microdialysis for continuous glucose measurement in vivo.

REFERENCES
