MICROFLUIDIC CARTRIDGE FOR (BIO)CHEMICAL FUNCTIONALIZATION OF PT MICROELECTRODES INTEGRATED ON SILICON MICROPROBES
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ABSTRACT
A microfluidic approach for the local deposition of different functional enzyme-membranes on Pt microelectrodes integrated on silicon microprobes is presented. To achieve this the electrode on the microprobe is placed in a microfluidic channel, which is subsequently flushed by the cross-linking enzyme solution using integrated pneumatic pumps. The simple and disposable cartridge can replace heavy dispensing equipment commonly used for this purpose.

KEYWORDS: Choline, Glucose, Microelectrode, Biosensor

INTRODUCTION
Development of amperometric multi-biosensors requires a sequential modification of different electrodes with different sensing layers. This implies a selective, spatially well controlled modification of certain electrodes while keeping the others unmodified. Currently, mostly dispensing is being used with, for decreasing sizes of electrodes, greatly increasing constraints on the volumes required [1].

In our work, silicon microprobe biosensors comprising two Pt electrodes of 50 µm x 150 µm, separated by 200 µm are being developed for monitoring of different biochemical compounds in the brain (fig. 1). Taking into account the 10 µm recess of the electrodes, the corresponding volume to be dispensed is 75 pl.

The objective of our work is to develop a family of microfluidic devices to be used at different phases of biosensor preparation and testing. We have presented recently a simple, disposable microfluidic device allowing fast and reproducible two-point calibration of a biosensor [2].

Here we describe another application of microfluidics to perform sequential depositions of different enzymatic layers on the Pt microelectrodes by glutaraldehyde co-crosslinking of the enzyme (e.g. choline oxidase) and bovine serum albumin (BSA).

Figure 1. Tip of the 6.5 mm long microprobe (100 x 100 µm² cross-section) with two integrated tip-electrodes (recessed by 10 µm).
DESIGN AND FABRICATION

The schematic layout of the cartridge allowing depositing two enzymatic membranes (glucose and choline oxidase) is shown in figure 2. It mainly consists of four channels i) two deposition channels (100 µm wide and 200 µm high) that are enlarged in their central part to form deposition chambers, ii) two drying channels and chambers (idem) and iii) three pneumatic pumps (A, B and C).

![Diagram](image)

**Figure 2. Schematic layout of the PDMS cartridge**

The first electrode on the microprobe is perpendicularly introduced via the insertion channel in the upper deposition chamber which is flushed with the first enzymatic solution using the integrated micropump A. Then it is transferred into the adjacent drying chamber. Due to the presence of the brushing structures, the sidewalls of the probe are cleaned resulting in a well delineated enzymatic membrane in the Pt microelectrode recess (fig. 3). For the deposition of the next membrane the second electrode is transferred into the second deposition channel, where the cycle is repeated. Micropumps B and C are used in this case. The pumping volume is calculated in a way that the required volume is reliably displaced even when simple screws as actuators operated by hand are used.

![Image](image)

**Figure 3. Removing the probe from the deposition channel leaves a clearly visible and precisely located solution drop in the bottom electrode recess.**

![Image](image)

**Figure 4. Cartridge placed in the set-up controlling microprobe alignment and insertion. Screws are used for the external actuation of the integrated micropumps.**
The disposable cartridges are fabricated by casting of poly(dimethylsiloxane) (PDMS) in a SU-8 mold. Following an oxygen-plasma activation, the PDMS structure is bonded on a glass slide to seal the channels and pumping chambers. The cartridge is then placed in a hand-held set-up comprising actuation screws and the positioning site for the silicon microprobe (fig. 4).

RESULTS AND DISCUSSION

A fluorescence image of the electrodes with respectively glucose oxidase and choline oxidase membranes is shown in figure 5. The biosensors response to consecutive additions of 25 µM and 10 µM of glucose and 25 of µM choline is illustrated in figure 6. The sensitivities obtained are similar to that of single biosensors. No cross-talk between the two sensors is observed due to the good spatial control of the enzymatic membrane deposition.

CONCLUSION

The elastomeric quality of PDMS allowed us to fabricate a pair of disposable cartridges for the functionalization and calibration of silicon microprobes. It is realized using an integrated pump system actuated by hand, resulting in a laboratory-independent, small set-up for on-site employment.

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REFERENCES