Smart SU-8 Pillars Implemented in a Microfluidic Bioreactor for Continuous Measurement of Glucose

Journal Item

Available online at www.sciencedirect.com
ScienceDirect
Procedia Engineering 5 (2010) 448–451
Procedia Engineering
www.elsevier.com/locate/procedia

Proc. Eurosensors XXIV, September 5-8, 2010, Linz, Austria

Smart SU-8 Pillars Implemented in a Microfluidic Bioreactor for Continuous Measurement of Glucose

S. Talaei[a]*, O. Frey[a], S. Psoma[b], P. D. van der Wal[a], N. F. de Rooij[a]

[a]*Ecole Polytechnique Fédérale de Lausanne (EPFL), Institute of Microengineering, Sensors, Actuators and Microsystems Laboratory Rue Jaquet-Droz 1, 2000 Neuchâtel, Switzerland*
[b]*University of Western Macedonia, Department of Engineering of Information Technology and Telecommunications Kozani 50 100, Greece*

Abstract

In this contribution we explore a new and simple approach for immobilizing enzymes like glucose oxidase on SU-8 surfaces to develop a smart substrate integrated in microfluidics. SU-8 is a well known photoresist often used in microfluidic prototyping. Immobilization of enzymes on such substance can open new possibilities in the microfabrication of enzyme biosensors and bioreactors. To demonstrate the consistency of this approach, we describe the design, fabrication and the simple functionalization of a microfluidic bioreactor employing smart SU-8 pillars for continuous amperometric measurement of glucose. The results reveal the possibility of simply binding enzymes on SU-8 surface. Moreover, a significant improvement in the linear response range is observed compared to the previous published amperometric microfluidic glucose sensors [1,2].

Keywords: SU-8; Enzyme; Bioreactor; Smart substrate; Glucose oxidase; Microfluidics; Glucose measurement; Microfabrication

1. Motivation

In our previous work [3] we introduced a technique for binding PDMS to SU-8 by treating the former with an oxygen-plasma and the latter with APS. The reasoning behind the treatment with APS was to bind any possible unreacted epoxy groups in SU-8 with NH_2 from APS. The positive results prompted us to investigate this reaction for other compounds. In the current research, we study the binding of free NH_2 groups of enzymes to SU-8 for biosensing applications. In this paper, we present our novel method for binding GOx to SU-8. This simple immobilization can be an alternative for enzyme entrapment in a crosslinked membrane.

* Sara Talaei. Tel.: +41-32-720-5432; fax: +41-32-720-5711.
E-mail address: sara.talaei@epfl.ch.

1877-7058
doi:10.1016/j.proeng.2010.09.143

Nomenclature

GOx	glucose oxidase
APS	3-aminopropyltriethoxysilane
PBS	phosphate buffer saline
n	number of experiments

2. Experimental

For evaluation of the technique, a microfluidic cartridge for detecting glucose, lactate and other analytes which are detectable by specific relevant enzymes is designed and fabricated. The goal is to immobilize the enzymes directly on SU-8 surface, without employing specific membranes for their stabilization. The schematic design is shown in Fig. 1. The cartridge is created in SU-8 and PDMS on a glass substrate. It includes a flow-through microchannel that directs the sample to a bioreactor and two platinum microelectrodes situated down-stream for amperometric detection. The area of the working and pseudo-reference/counter electrodes are 3.2 mm^2 and 5.6 mm^2 respectively. The enzyme is immobilized in the bioreactor including 84 SU-8 pillars in the area of 15.6 mm^2. The diameter of the pillars is 240 µm and their height is 75 µm. Enzyme immobilization is done by simply dispensing of 5 µl of a solution made of 6 mg GOx and 250 µl Triton X-100 (3 g/l dilution) in 250 µl DI-water in the designated section of the microchannel containing the pillars. Triton X-100 is a non-ionic surfactant for better wetting of the hydrophobic SU-8 surface. After drying (~ 5 min), the structured PDMS layer was placed on top. The PDMS microchannel has a width of 800 µm and a height of 200 µm. The cartridges were stored dry at 4 °C when not in use.

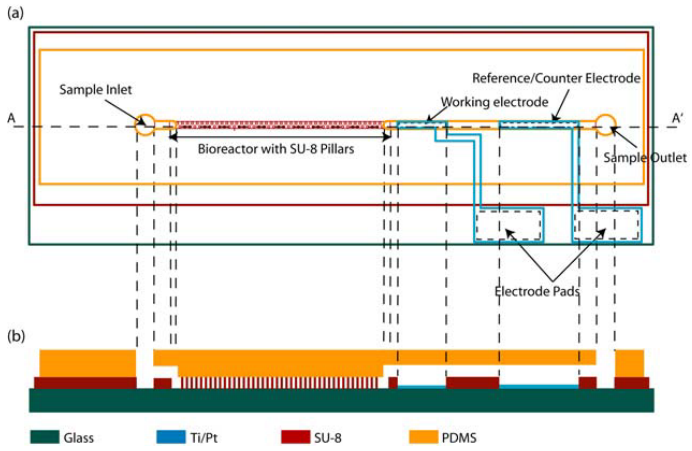
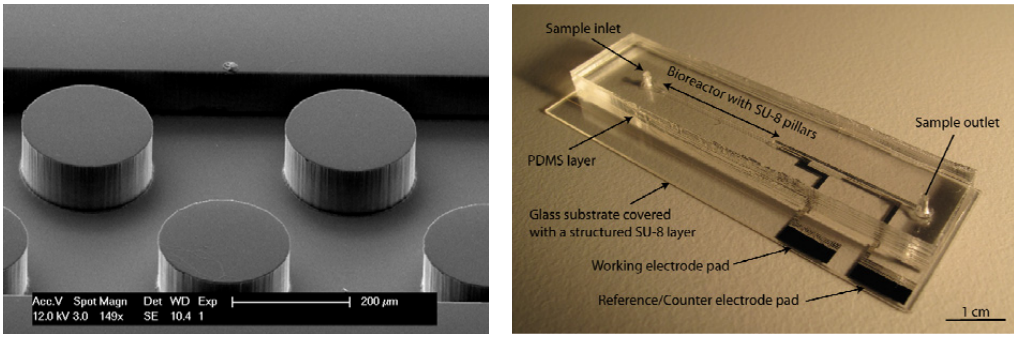


Fig. 1: Microfluidic cartridge design. (a) Top-view of the microfluidic cartridge (b) Cross-section of the microfluidic cartridge along AA'

Fig. 2.a is a SEM image of the SU-8 pillars inside the bioreactor. The pillars increase the surface area onto which the enzyme can bind. The completed cartridge which is shown in Fig. 2.b was placed in a chip holder with fluidic and electrical connections. GOx converts glucose and oxygen into gluconic acid and hydrogen peroxide. The peroxide can be detected amperometrically.

Before measurements, the channel was washed with a continuous flow of PBS for 20 minutes to remove any unbound enzyme. For characterization of the bioreactor, 3 similar cartridges were tested under the same conditions.

The working electrode was polarized at 0.5 V versus the pseudo reference/counter electrode. Glucose solutions with different concentrations were pumped into the cartridge with controlled flow-rates by means of a standard syringe pump.



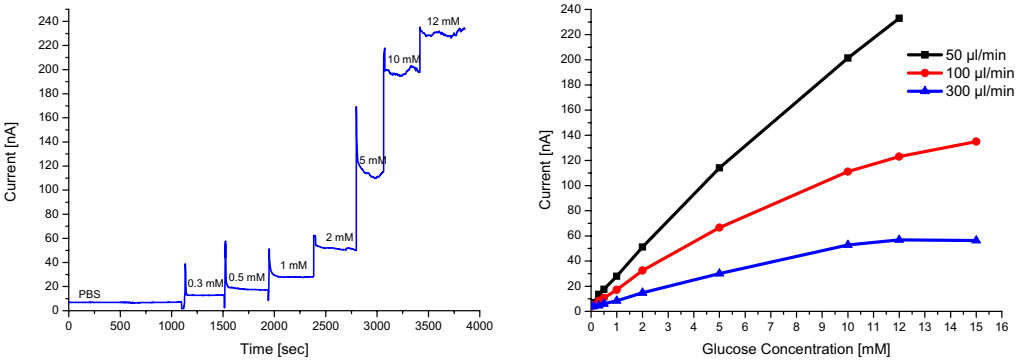
(a) (b)

Fig. 2: a) SEM photograph of the smart SU-8 pillars in the bioreactor b) Picture of the fabricated microfluidic cartridge

3. Results and discussions

A typical response of the working electrode when glucose was pumped through the bioreactor is shown in Fig. 3.a. By increasing the flow-rate of the sample, a decrease was observed in the sensitivity of the bioreactor. Fig. 3.b is demonstrating that the sensitivity of the bioreactor is adjustable with the flow-rate of the sample. The sensitivity was 33 ± 11 nA/mM among three tested bioreactors.

The linear response range of the bioreactor [Fig. 3] was approximately 10 mM which is 10 times more than the previously reported microreactor with immobilized enzyme on glass beads [1], and 5 times more than the cartridge in which GOx was immobilized in a membrane formed by crosslinking on top of the electrode [2]. Furthermore, the presented effective immobilizing technique eliminates the application of toxic chemical products like glutaraldehyde that is used frequently in enzymatic membranes.



(a) (b)

Fig. 3: a) Glucose bioreactor calibration curve at sample flow-rate of 50 µl/min, 8 days after functionalization of SU-8 pillars. The linear response range was up to 10 mM b) Glucose bioreactor calibration curve at three different sample flow-rates 8 days after functionalization. By increasing the flow-rate from 50 µl/min to 300 µl/min, the sensitivity decreased from 19.5 nA/mM to 4.9 nA/mM

The calibration curves of the bioreactor during the first 49 days after functionalization are shown in Fig. 4 while different glucose concentrations were pumped into the cartridge at constant flow-rate of 50 µl/min.

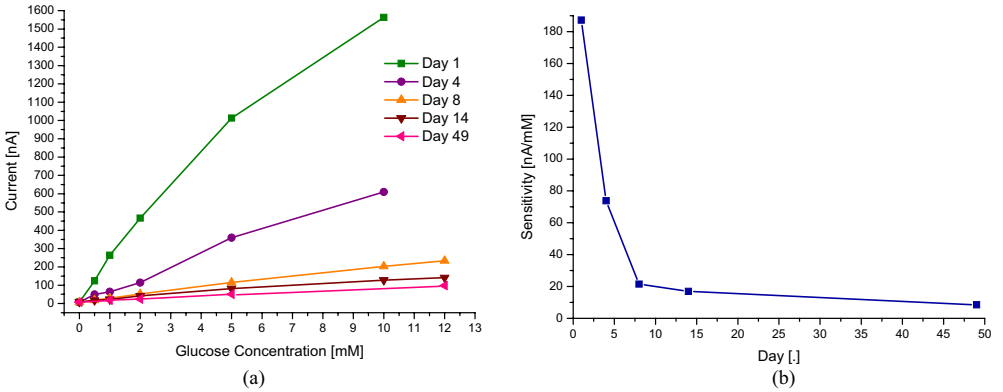


Fig. 4: a) Glucose bioreactor calibration curve over time. The samples were pumped into the cartridge at constant flow-rate of 50 µl/min. b) Sensitivity of the glucose bioreactor upon the linear response range over a period of 49 days

The graphs in Fig. 4 are presenting a considerable decrease in sensitivity of the bioreactors especially during the first week. Further experiments are in progress to investigate whether this decrease is a result of natural degradation of the enzyme or loss of stability in enzyme binding to the SU-8 surface.

4. Conclusions

A simple method for immobilizing GOx on a SU-8 surface is presented in this paper. This technique is employed in a microfluidic cartridge to stabilize the enzyme on micro-scale SU-8 pillars forming a smart substrate acting as a bioreactor for continuous measurement of glucose. The results of experiments demonstrate that non crosslinked epoxy groups in SU-8 can easily bind to NH_2 groups of the enzyme, and the bioreactor was active for measuring glucose concentration at least for 49 days after functionalization. The sensitivity of the bioreactor was 33 ± 11 nA/mM (n=3), and the linear response range was close to 10 mM.

Acknowledgements

The authors gratefully thank the staff at the Microsystems Technology Division of the CSEM.

References

[1] D. J. Strike et al., “Enzymatic microreactor using Si, glass and EPON SU-8”, Biomedical Microdevices, 2 (2000) 175-178

[2] O. Frey et al., “Disposable, continuous-flow biosensor for multi-analyte monitoring and microfluidic control of the linear range”, Proceedings of µTAS XII, USA, (2008) 116-118

[3] S. Talaei et al., “Hybrid microfluidic cartridge formed by irreversible bonding of SU-8 and PDMS for multi-layer flow applications”, Procedia Chemistry, 1 (2009) 381-384