Sensors and Actuators B 216 (2015) 434–442

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

journal homepage: www.elsevier.com/locate/snb

# Disposable electrochemical sensor prepared using 3D printing for cell and tissue diagnostics

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ARTICLE INFO

*Article history:*
Received 24 December 2014
Received in revised form 11 April 2015
Accepted 13 April 2015
Available online 29 April 2015

*Keywords:*
Bioelectrochemistry
PDMS sensor
3D printing

ABSTRACT

In this paper we present a novel electrochemical sensor with a unique 3D architecture allowing for direct measurements on contact, or in close proximity, to biological samples. For biomedical applications, the all-polymer architecture can be mounted on special probes that can access the region under test with no need for biopsy as is done today with the conventional 2D electrodes. The chip consists of a biocompatible substrate comprised of an electrochemical cell with two gold electrodes (working and counter) and an Ag/AgCl quasi-reference electrode. The metal electrodes on the biochip front (sensing) side are fabricated by conventional electroplating and patterning methods. The chip itself is made from PDMS cast from a polymer master fabricated by 3D printing. The electrical communication between the biochip front and backside is enabled by through-hole *via*-contacts filled with conductive PDMS containing 60 wt% graphite powder. The electroactivity of working electrodes was verified by cyclic voltammetry of ferrocyanide/ferricyanide redox reaction. Amperometric *in-vitro* detection of the biomarker alkaline phosphatase from three different colon cancer cell lines directly in a cell culture plate while maintaining their biological environment was successfully demonstrated. The sensor exhibit stable voltammetric signatures and significant amperometric response to the enzyme in repeated tests. This approach paves the way to perform direct, non-invasive diagnostics on top of an exposed cell layer for both *in-vivo* and *in-vitro* applications.



## 1. Introduction

Electrochemical sensors allowing for the monitoring of cell vitality are of prime interest to the medical and healthcare community. One of the key challenges for such sensors is the positioning of the sensing elements into intimate contact with or in the immediate vicinity of the cells or tissues under test. Significant efforts have been made to develop non-invasive tools for cellular microphysiological monitoring [1,2]. The applications of these methods extend from clinical tests to research in areas such as cancer, neurobiology and pharmacology to environmental monitoring [3–7]. An appealing approach for real-time monitoring of living cells is to carry out the relevant measurements at the immediate apical surface of the targeted cells. This approach offers several fundamental advantages: short diffusion distance of secreted analytes towards the electrode surface improving signal to noise ratio, reducing response time, enhancing analytical performance and increasing the sensitivity [8,9]. Noninvasive, vertical detection approach from cells has been mainly utilized to investigate exocytotic activity of neurotransmitters [10,11]. Advances design in microelectrode arrays (MEAs) including imaging techniques allows detection down to a single-cell [12,13]. In addition, detection of metabolic events by scanning electrochemical microscopy (SECM) was also reported [14,15]. Although, SECM is a useful tool for single-cell studies it was designed for research purposes and cannot be used as a portable diagnostic device.

In the commonly used three integrated electrode cell (with reference, working and auxiliary electrodes on the same substrate *e.g.* screen-printed electrode) the sensing device and the connections to the measurement device are located on the same side of the substrate. Such devices require the tedious process of cells or tissue detachment from their culture or tissue origin and reattachment onto the electrode surface often reducing cell response and lowering the signal to noise ratio [16,17]. Furthermore, the

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http://dx.doi.org/10.1016/j.snb.2015.04.065
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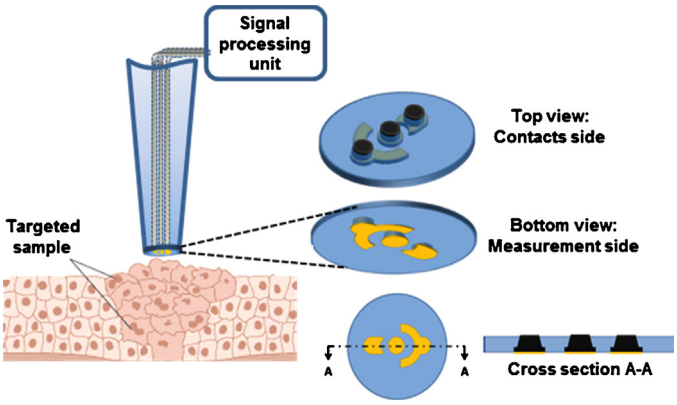


**Fig. 1.** Schematic representation of the direct diagnosis detection method on cells.

response of electrochemical biosensors is often limited by diffusion limitations. In large systems, agitation or stirring introduces convection accelerating the transport towards the electrodes. However, in miniature or micron scale systems the ability to introduce convection is limited. As such, small scale systems are expected to benefit from a reduction in dimension and distance between the sampled tissue or cells and the electrodes.

These limitations can be overcome using a three-dimensional (3D) sensing system architecture where the sensing electrodes and the electrical contacts are positioned vertically on the opposite sides of the same chip (Fig. 1). This allows positioning of the electrodes to an intimate contact with, or in close proximity to the tissue or cells under test. Such designs can also benefit from the versatility of 3D printing for customized sensor design tailored to an organ or tissue size and shape. Another advantage of the use of flexible substrate is the reduction in mechanical stress facilitating improved attachment to the organ or tissue.

The establishment of a 3D sensor of such design on a flexible substrate is challenging due to material and process constraints. For example, *vias* cannot be sufficiently etched through a PDMS substrate using standard wet or dry etching techniques. Furthermore, conventional interconnect patterning of microelectrodes systems includes metal and dielectric layers deposition and patterning steps [18–20]. Such process typically has several lithography and deposition steps that are usually performed in a clean room environment. Such microfabrication techniques are complicated and require complex infrastructure and expensive equipment.

3D printing provides new means for the integration of active elements on and into elastomeric substrates, offering an alternative to the largely incompatible standard microfabrication techniques like photolithography and wet and dry etching. The key role of 3D printing is one-step fabrication of 3D complex shaped polymer elements [21]. The concept of 3D printing was introduced over 30 years ago; since then, various techniques were developed and applied in many areas, particularly in biomedical applications and engineering. 3D printers build a device layer by layer based on a 3D computer models. This technique can be applied for mass production in a short time with relatively low engineering effort and high yield. Thus 3D printing was successfully applied for the fabrication of 3D electrode for electrophysiological recording [22] and microfluidic polymer devices [23].

In this paper, we report design, fabrication and feasibility demonstration of a portable and disposable electrochemical sensor for 'downwards' sensing of metabolites or secreted biologically active molecules. The sensor capability of rapid detection of the biomarker alkaline phosphatase (ALP), secreted from colon cancer cell lines was demonstrated. This example highlights the sensor applicability for robotic testing of arrays of cell lines or tissues

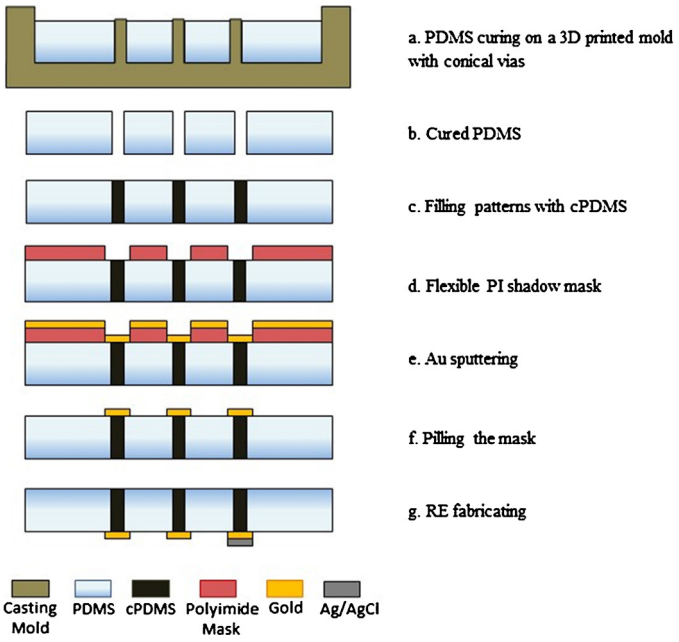


**Fig. 2.** Schematic diagram of the 3D sensor fabrication process.

without damaging (or otherwise affecting) the cells while providing immediate information on cell physiology.

This 3D structure allows for miniaturization down to the micron scale and will pave the way to perform direct, non-invasive diagnostics on top of an exposed cell layer for both *in-vivo* and *in-vitro* applications.

## 2. Experimental

### 2.1. Sensor design

The flexible and biocompatible chip is composed of an electrochemical cell with two gold electrodes (working and counter) and Ag/AgCl quasi-reference electrode. The sensor is enclosed in a portable, 3D printed housing which provides mechanical support and allows controllable movement in three dimensions. A schematic representation of this design for the direct diagnosis on cells can be seen in Fig. 1; the electrodes are placed on the lower side, facing the exposed surface of the sample tested, wherein the signals are routed to the opposite side of the chip *i.e.* the side not in contact with the sample. Transfer of a signal from the electrodes to the back side of a chip proceeds by means of the specially designed through-substrate *via* contacts. Conventional processes for filling through substrate *vias* commonly relies on copper electroplating which is a complex, time-consuming and expensive procedure [24,25]. In the present work, the *vias* were filled with a conductive carbon-polymer composite resulting in a 3D "all-polymer" electrochemical sensor. Schematic diagram of the 3D sensor fabrication process is presented in Fig. 2. The consecutive stages of chip fabrication are shown in Fig. 3.

### 2.2. Mold fabrication

The initial negative molds (Fig. 3a) were designed in SolidWorks® and fabricated using stereolithography (Objet Connex500™ 3D printing system) from a proprietary polymer, a rigid white material (VeroWhitePlus™). The molds were then cleaned in a 2% NaOH solution for at least 30 min (Fig. 3b).