Electrochimica Acta 246 (2017) 234–241

Contents lists available at ScienceDirect

Electrochimica Acta

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

Differential imaging of the metabolism of bacteria and eukaryotic cells based on light-addressable potentiometric sensors

Shahriar Dantism[a,c], Shoko Takenaga[a], Torsten Wagner[a,b], Patrick Wagner[c], Michael J. Schöning[a,b,*]

[a] Institute of Nano- and Biotechnologies (INB), Aachen University of Applied Sciences, Campus Jülich, 52428 Jülich, Germany
[b] Peter Grünberg Institute (PGI-8), Research Centre Jülich GmbH, 52425 Jülich, Germany
[c] Department of Physics and Astronomy, Soft-Matter Physics and Biophysics Section, KU Leuven, Celestijnenlaan 200 D, 3001 Leuven, Belgium

ARTICLE INFO

Article history:
Received 31 January 2017
Received in revised form 29 May 2017
Accepted 30 May 2017
Available online 3 June 2017

Keywords:
LAPS
extracellular acidification
Escherichia coli K12
Chinese hamster ovary (CHO) cells
3D-printed multi-chambers

ABSTRACT

A light-addressable potentiometric sensor (LAPS) is a field-effect-based potentiometric sensor with an electrolyte/insulator/semiconductor (EIS) structure, which is able to monitor analyte concentrations of (bio-)chemical species in aqueous solutions in a spatially resolved way. Therefore, it is also an appropriate tool to record 2D-chemical images of concentration variations on the sensor surface. In the present work, two differential, LAPS-based measurement principles are introduced to determine the metabolic activity of *Escherichia coli* (*E. coli*) K12 and Chinese hamster ovary (CHO) cells as test microorganisms. Hereby, we focus on i) the determination of the extracellular acidification rate (ΔpH/min) after adding glucose solutions to the cell suspensions; and ii) recording the amplitude increase of the photocurrent (I_{ph}) related to the produced acids from *E. coli* K12 bacteria and CHO cells on the sensor surface by 2D-chemical imaging. For this purpose, 3D-printed multi-chamber structures were developed and mounted on the planar sensor-chip surface to define four independent compartments, enabling differential measurements with varying cell concentrations. The differential concept allows eliminating unwanted drift effects and, with the four-chamber structures, measurements on the different cell concentrations were performed simultaneously, thus reducing also the overall measuring time.

1. Introduction

Studies on the metabolism of living cells and microorganisms using field-effect-based (bio-)sensors have gained increasing attention by numerous research groups working in the interdisciplinary fields of microbiology, cell-culture technology, bioengineering, microbial engineering, and nanotechnology. This class of sensors allows a low-cost and fast process monitoring, which is important in various applications: A few examples include studies on a broad field of applications in biochemical and biological systems [1,2], environmental (bio-)sensing [3], and some specific field of applications such as cell-based biosensors [4–6], investigations on cellular metabolism [7–10], biosensors for neurophysiological measurements [11], detection of penicillin and antibiotics [12,13], label-free detection on DNA and tumor markers [14,15], and high-throughput analytical screening on living cells [16].

Another important approach in the field of renewable energy is the on-line monitoring of the metabolic activity of microorganisms involved in the intermediate stages (*e.g.*, acidogenesis, acetogenesis) of biogas production in fermentation reactors. Here, it is the purpose to detect possible process disturbances by recording the metabolic activity of microorganisms during the operation. This should improve not only the quantity and quality of the produced methane gas at the end of the process but also reduce related costs due to irreversible collapse of the process [17]. In addition, the yield of the large-scale fermentation processes in the pharmaceutical industry will benefit from a direct measurement of the metabolic activity of the utilized microorganisms. In this context, a light-addressable potentiometric sensor (LAPS) can be employed as a suitable tool enabling investigations on cellular metabolisms of microorganisms. A LAPS belongs to the group of field-effect-based potentiometric sensors with an electrolyte/insulator/semiconductor (EIS) structure. As a special benefit, a LAPS is able to monitor analyte concentrations of (bio-)chemical species in an aqueous solution in a spatially resolved manner [18].

* Corresponding author at: Institute of Nano- and Biotechnologies (INB), Aachen University of Applied Sciences, Campus Jülich, 52428 Jülich, Germany.
E-mail address: schoening@fh-aachen.de (M.J. Schöning).

http://dx.doi.org/10.1016/j.electacta.2017.05.196
0013-4686/

Therefore, it is also appropriate to record 2D-chemical images of the concentration variations on the sensor surface [19–24]. Considering these abilities, the main motivation of our work is to introduce two possible experimental LAPS-based methods to determine the cellular metabolism of two commonly used and easy to cultivate test microorganisms: 1) investigation of the extracellular acidification rate (ΔpH/min) of *E. coli* K12 cells applying a multi-chamber differential LAPS system. The system utilizes an array of 16 light-emitting diodes (LEDs). Four LEDs were assigned to each chamber. It was steered by a field-programmable gate array (FPGA)-based setup [25]; 2) recording the increase of the amplitude of the photocurrent (I_{ph}) related to the acidification of *E. coli* K12 bacteria and CHO cells on the sensor surface with a 2D-differential imaging approach using a moveable laser diode and multi-chambers. Regarding these goals, 3D-printed four-chamber structures were developed and combined with LAPS chips, which enable differential and simultaneous measurements without additional immobilization steps [8,9].

2. Experimental

2.1. LAPS set-up and functional principle

A schematic illustration of a differential LAPS measurement system is depicted in Fig. 1. The design of a LAPS chip can be described as a multi-layer structure comprising i) a p-doped ⟨100⟩ silicon substrate (540 μm thickness, resistivity of 5–10 Ωcm), ii) an insulator layer (SiO_2, 30 nm thick), iii) a pH-sensitive transducer layer (60 nm Ta_2O_5) with nearly-Nernstian pH sensitivity of 54 mV/pH [26], and iv) an Ohmic rear-side contact (Al, 300 nm). The pH-sensitive transducer layer is in direct contact with the analyte solution. The chip size is 20 mm × 20 mm each, with a sensor-active area of 15 × 15 mm^2 for the light source to illuminate the rear side. More information on the processing steps for LAPS-chip preparation can be found in [27]. The working principle of a LAPS-based sensor can be shortly summarized as follows: A positive bias voltage (V_{bias}) between an Ag/AgCl reference electrode in the liquid (RE-1S, SG137 ALS Co., Ltd) and the Ohmic rear-side contact generates a depletion region (space-charge region) at the semiconductor/insulator (p-silicon/SiO_2) interface. By means of a modulated (1 kHz) light source (an array of light-emitting diodes or a moveable laser diode) with the light beam entering through the backside of the chip, electron-hole pairs are generated, which can be separated in the electric field of the space-charge region. For a bias-voltage sweep from negative to positive voltages, a typical sigmoidal-like photocurrent-voltage (I–V) curve can be recorded [28]. By increasing the H^+-ion concentration in the analyte solution, a corresponding shift of this I–V curve to the negative bias-voltage axis can be observed. Conversely, a decrease of the H^+-ion concentration results in a related potential shift to the positive bias-voltage axis.

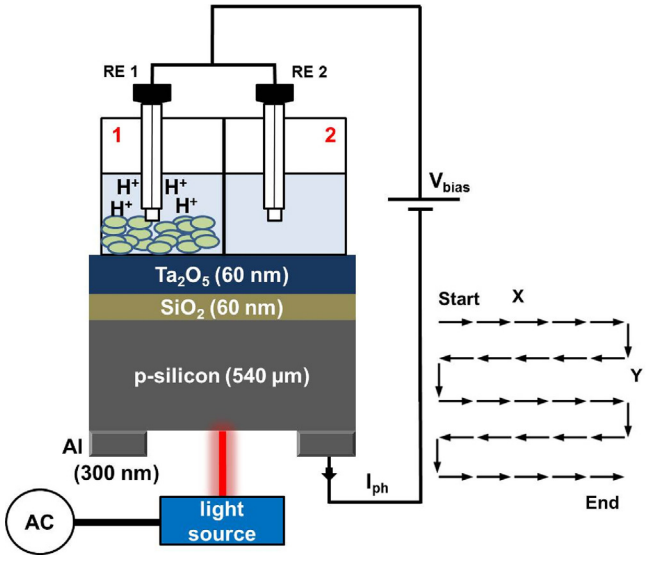

Fig. 1. Schematic illustration of the multi-chamber differential measurement set-up based on the LAPS principle. Active sensor side with cells in PBS buffer (1), reference sensor side with PBS buffer without cells (2). The sensor chip consists of an Al/p-Si/SiO_2/Ta_2O_5 structure. RE: reference electrode (L = 52 mm, Ø = 4.5 mm, filled with 3 M NaCl), I_{ph}: photocurrent, V_{bias}: bias voltage. Applied light sources: a moveable laser diode scanning in x-y direction or an array of 16 light-emitting diodes (LEDs).

2.2. Development of 3D-printed multi-chamber structures

3D prototypes with different geometries were constructed using computer-aided design (CAD) software (Autodesk inventor 2015). The digital models were printed applying a photo-polymer (polypropylene-acrylonitrile-butadiene-styrene, PP-ABS) in an UV-LED 3D-printer (Freeform Pico Plus27, Asiga). The printing principle is based on stereolithography and layer-by-layer technology. Subsequently, after finishing the printing procedure (approx. 2 h), each individual printed structure was hardened in an ultraviolet (UV) oven for approx. 20 min. Following this stage, the measurement chambers were fixed on LAPS chips defining four independent compartments using 50 μl of a liquid transparent adhesive silicon rubber (MOMENTIVE™). The bonded chambers on LAPS chips were dried at 25 °C for approx. 3 h. The four-chamber design allows simultaneous measurements with different cell concentrations (the fourth chamber serves as reference chamber without cells). Moreover, correcting for certain variations of the cell concentrations and more experimental flexibility than for the differential two-chamber approach described in our previous work is possible [27].

Fig. 2 shows two designs of printed four-chamber structures on chip holders to be placed on a light source (array of 16 LEDs or a laser diode, moveable in x-y direction for 2D-chemical imaging). Due to the geometry of developed multi-chamber structures, utilizing a rectangular arrangement has provided differential measurements with the LAPS setup applying four LEDs in each chamber (see Fig. 3) improving the rear-side illumination compared to the triangular arrangement, which was mainly designed initially for measurements with the scanning LAPS setup. Furthermore, it was found, that the rectangular arrangement enables a better analyte distribution on the sensor surface within chambers, which was taken into consideration for

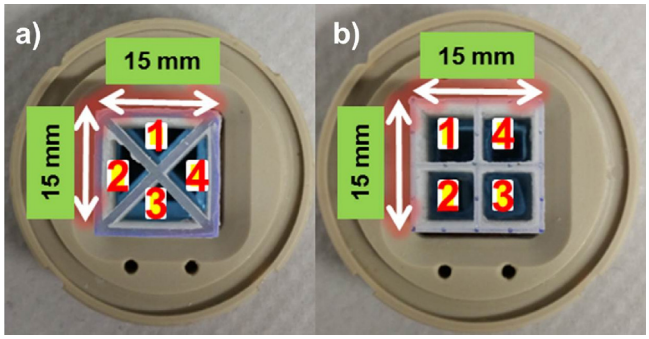

Fig. 2. Images of the developed multi-chamber structures mounted on the LAPS chip by using adapted chip holders (polyether ether ketone (PEEK), diameter: 40 mm, height: 10 mm) a) four-chamber triangular arrangement (height: 20 mm, wall thickness: 1.2 mm, volume/chamber: 600 μl), b) four-chamber rectangular arrangement (height: 20 mm, wall thicknesses: 1 mm, volume/chamber: 800 μl).