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Light-addressable potentiometric sensor (LAPS) combined with magnetic beads for pharmaceutical screening

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ABSTRACT

The light-addressable potentiometric sensor (LAPS) has the unique feature to address different regions of a sensor surface without the need of complex structures. Measurements at different locations on the sensor surface can be performed in a common analyte solution, which distinctly simplifies the fluidic set-up. However, the measurement in a single analyte chamber prevents the application of different drugs or different concentrations of a drug to each measurement spot at the same time as in the case of multi-reservoir-based set-ups. In this work, the authors designed a LAPS-based set-up for cell culture screening that utilises magnetic beads loaded with the endotoxin (lipopolysaccharides, LPS), to generate a spatially distributed gradient of analyte concentration. Different external magnetic fields can be adjusted to move the magnetic beads loaded with a specific drug within the measurement cell. By recording the metabolic activities of a cell layer cultured on top of the LAPS surface, this work shows the possibility to apply different concentrations of a sample along the LAPS measurement spots within a common analyte solution.

1. Introduction

1.1. Principle of the light-addressable potentiometric sensor (LAPS)

The LAPS belongs to semiconductor-based sensors utilising the interaction of a particular analyte with a transducer layer by forming a Nernstian potential, and the influence of this interaction on the underlying space-charge region [1,2]. Fig. 1a) illustrates the principle of a typical LAPS. Variations of a specific analyte in the solution will generate a change in the surface potential of a transducer layer (e.g., Ta_2O_5 as a pH-sensitive material). These additional local variations of the surface potential will be superimposed with the applied bias voltage and hence, modify the underlying space-charge region, located at the insulator-semiconductor interface.

To determine the change of the space-charge region, a modulated light beam is used to illuminate a certain area of the LAPS structure. The light beam will create electron-hole pairs, separated within the electric field in the semiconductor, resulting in an externally detectable photo current. Since the amplitude of the photo current is mainly affected by the local changes of the illuminated area, this method provides a laterally resolved measurement of the analyte concentration close to the sensor surface. By focusing the light beam to different regions of the semiconductor structure, it is possible to define different measurement spots on the sensor surface.

By sweeping the applied bias voltage and simultaneously recording the resulting photo current, one can obtain typical current *vs.* voltage plots (I/V curves). As shown in Fig. 1b), depending on the local concentration of the analyte, the I/V curve moves along the horizontal axis. By defining a working point for a fixed photo current I_{WP} (e.g., at the inflection point of the slope), one can calculate the horizontal shift ΔV, which correlates to the local change of the Nernst-like potential at this particular measurement spot. An initial calibration can be used to map those bias voltage shifts to a certain change of the analyte concentration in the

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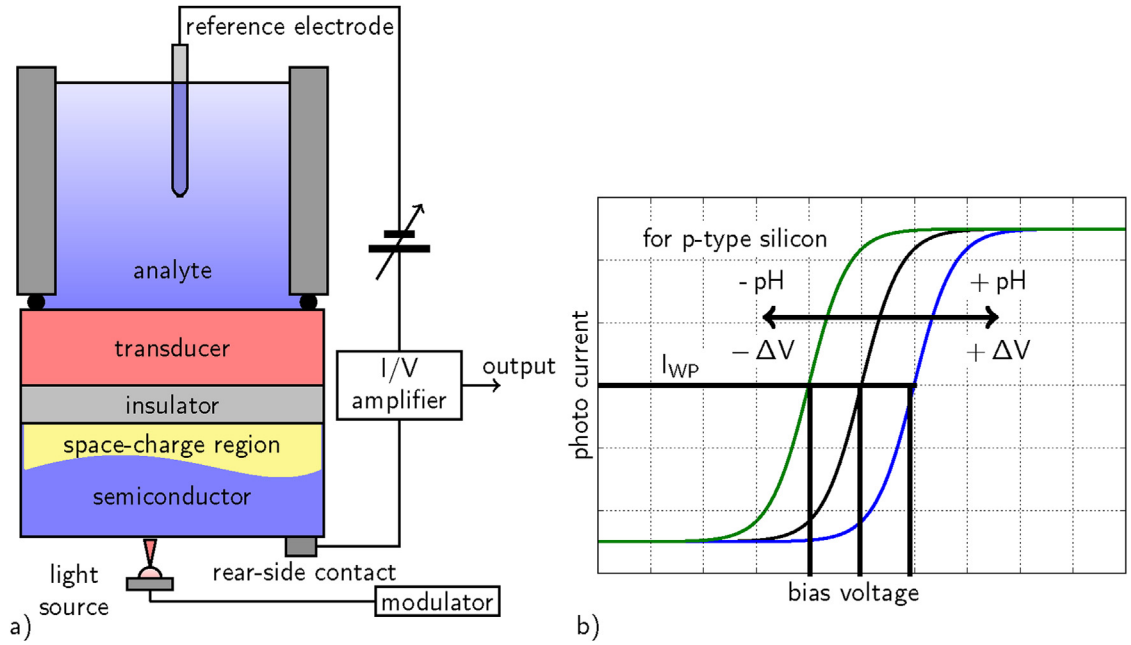

Fig. 1. a) Schematic representation of a typical LAPS set-up. A bias voltage is applied across the LAPS structure to induce a space-charge region. A modulated focused light beam illuminates a certain region of the LAPS structure from below. A trans-impedance amplifier is used to record the generated photo current. b) Schematic I/V curves of a LAPS structure for different analyte concentrations. The horizontal shift is proportional to the change of the analyte concentration.

solution. More details of the measurement principle can be found in literature [3–9].

One promising application of LAPS devices is the combination with an additional cell layer on top of the transducer layer as shown in Fig. 2a). The authors demonstrated the use of various LAPS set-ups to determine the metabolic activity of CHO (Chinese hamster ovary) cell cultures at up to 16 measurement spots simultaneously [10–12] within a cell culture flask. Carcinogenic cells metabolise the surrounding culture medium and one of the final metabolic steps is the formation of an acid. This will cause a pH shift that is detectable by the underlying pH-sensitive transducer structure. Depending on the condition and the reaction of the cells towards a particular environment, higher or lower changes in the pH value over a fixed period of time will be observed, corresponding to a higher or lower metabolic activity, respectively.

In this work, the authors present a method to observe the metabolic activity of a layer of macrophages at 16 individual measurement spots on a single LAPS structure. Furthermore, a method to stimulate individual measurement spots of a cell layer of macrophages by magnetic beads loaded with an endotoxin (lipopolysaccharides, LPS) is proposed. By applying the magnetic field at different locations, concentration gradients of the drug can be generated by attracting the magnetic beads along the magnetic field (see Fig. 2b)).

1.2. Interaction between lipopolysaccharides (LPS) and macrophages

To interpret the following measurement results, a brief description of the interaction between macrophages and LPS, as described in literature, will be given. The different characteristics in the activation and differentiation of macrophages are rather complex with the secretion of a large amount (>100) of different substances and an expression of about fifty different surface receptors on the cell surface [13]. Some products are constitutively secreted, whereas for others a selective induction of cell surface receptors is required.

Among all possible antagonists, LPS is one of the strongest activators of macrophages. The interaction between macrophages and LPS is characterised by the binding of LPS in the analyte solution to a specific LPS-binding protein (LBP) [14]. This causes an increase of the sensitivity of macrophages towards LPS. The resulting LPS-LBP complex binds to the protein CD14 on the cell surface, which facilitates the signal transduction [15]. So-called “toll-like receptors”, in that case esp. TLR4 associated with a MD-2 molecule, perform the final signal transduction. An increase in the metabolic activity had been identified by the production of TNF-α (tumor necrosis factor), nitric oxide and oxygen radicals. Higher concentrations of LPS even tend to cause a cytotoxic effect after some time [16]. Further detailed information of the signal chain of LPS on macrophages can be found elsewhere [17–22].

2. Materials and methods

2.1. Sensor set-up

The LAPS sensor consists of a boron-doped silicon wafer (350–400 μm, (100), 1–10 Ωcm), a 50 nm thick SiO_2 layer prepared by thermal oxidation and a 50 nm thick pH-sensitive Ta_2O_5 layer, deposited by PVD and subsequent thermal oxidation [23]. A 300 nm aluminium layer was sputtered on the rear side of the sensor structure and a thermal annealing step was performed to form an Ohmic rear-side contact. The wafer was diced into 2×2 cm^2 chips and the rear-side contact was partially removed to create an illumination window for the light beams. The LAPS chip was embedded in a plastic card to be inserted into a home-made reader unit. IR-LEDs, as light sources, are placed directly below the semiconductor chip in a 4×4 array shape [24]. Fig. 2b) depicts the position of the measurement spots 1–16 defined by the IR-LED array. Furthermore, the set-up provides the corresponding contacts for the rear-side contact of the LAPS structure, the sealing against the measurement cell on the LAPS surface and a connector for the