

Electrochemical microsensor system for cancer research on photodynamic therapy *in vitro*

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Abstract. An electrochemical microsensor system to investigate photodynamic therapy of cancer cells *in vitro* was developed and applied to monitor the cellular respiration during and after photodynamic therapy. The redox activity and therefore influence of the photodynamic drug on the sensor performance was investigated by electrochemical characterization. It was shown, that appropriate operation conditions avoid cross-sensitivity of the sensors to the drug itself. The presented system features a cell culture chamber equipped with microsensors and a laser source to photodynamically treat the cells while simultaneous monitoring of metabolic parameter *in situ*. Additionally, the optical setup allows to read back fluorescence signals from the photosensitizer itself or other marker molecules parallel to the microsensor readings.

1. Introduction

Photodynamic Therapy (PDT) is a two-step cancer treatment using a photosensitizing drug followed by local irradiation with visible light. PDT is a clinically established method to treat bladder, colon or skin cancer benefiting from the relatively small stress for the patient [1]. In the case of an insufficient treatment additional therapies, e.g. radiation therapy, are still applicable. Although PDT is applied clinically, the biochemical processes during and after the therapy are only partly understood [2]. Nowadays, cell models for the therapy are evaluated by single point (end point) measurements only, due to the lack of an appropriate *in vitro* monitoring platform to trace metabolic effects continuously. Thus transient effects, which are supposed to occur during the application in patients, will be overseen in the classical cell model. The proposed electrochemical sensor system enables the monitoring of changes in metabolism during and after the treatment in cell culture. Due to the integration of the sensor onto a transparent glass chip the parallel optical and electrochemical monitoring of the cells is enabled.

In this work, the photosensitizer drugs are characterized electrochemically with respect to possible interference with the metabolic monitoring. Afterward the system's performance is demonstrated monitoring oxygen concentration during and after photodynamic therapy in breast cancer cell culture. Recent developments brought up several microsystems enabling PDT *in vitro* along with optical readout [6-9]. In contrast, the presented system focuses on the combination of electrochemical sensors together with optical access, both also possible during the therapy itself.



2. Electrochemical Microsensor System

A platform to monitor the metabolic effects of cancer cells during and after PDT was developed. The system is based on a transparent sensor chip using electrochemical sensors [3, 4], integrated into an optical setup [5]. In this work, a chronoamperometric oxygen sensor was chosen as an example to demonstrate the feasibility of continuous measurement of cellular respiration. The transparency of the sensor chip enabled the optical inspection of the cells by an inverted microscope.

The sensor chip was embedded in a microsensor system. This arrangement allowed the cultivation of cells in a small chamber directly on the sensor chip, see figure 1 and 2.



Figure 1: Electrochemical sensor with cell culture area (in transparent plastic cylinder).

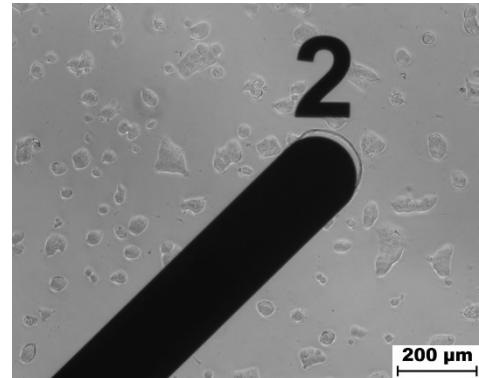


Figure 2: T-47D breast cancer cells 24 h after seeding on sensor chip next to the working electrode with connection line.

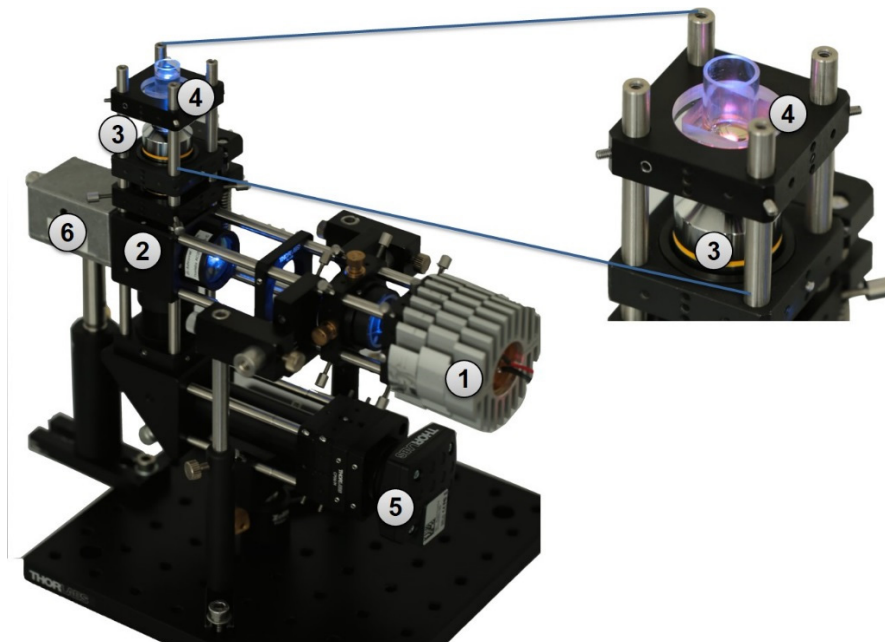


Figure 3: Setup for excitation of cell cultures with laser light for PDT featuring simultaneous detection of emitted fluorescence light and monitoring of metabolic parameters with electrochemical sensors. The light from the laser source (1) is deflected at a dichroic mirror (2) through the objective (3) to the cell culture chip (4). Fluorescence emission from the cell culture chamber passes through the dichroic mirror (2) and is detected by the CMOS-camera (5). The laser intensity is monitored by a detector (6).

The microsensor system was integrated into a setup together with a laser source for the photodynamic therapy and a fluorescence microscope, see figure 3. The beam from the diode laser (473 nm) was reflected at a dichroic mirror and reached the microsensor system through an objective lens. A CMOS camera at the end of the optical path allowed to detect fluorescence light emitted from the photosensitizer during the treatment. Additionally, this feature opened the possibility to use a fluorescence marker after the treatment.

3. Results

3.1. Electrochemical Investigation

An electrochemical investigation was performed to investigate the processes of the photosensitizer PpIX itself. PpIX was dissolved following an established protocol by using a solution named “Gomer” consisting of a mixture of perchloric acid, distilled water and methanol. The Gomer solution in a typical PpIX concentration of 70 μM results in a methanol concentration of 0.5 M. Since this is a considerable high concentration of methanol a cyclic voltammetric measurement, in the range of -0.6 V to 1.2 V was performed to investigate possible effects. The measurement was compared to a typical phosphate buffered saline (PBS) curve, see figure 4. It shows a prominent peak at 0.3 V, which is characteristic for the oxidation of methanol. The measurement was repeated with a 70 μM PpIX solution in PBS, once under normal oxygen atmosphere and once after the solution was purged with Nitrogen. The additional significant contribution due to the methanol oxidation was not visible anymore, see figure 5. In cyclic voltammetry experiments, no significant dependency of the redox behavior of the photosensitizer regarding the oxygen concentration can be observed.

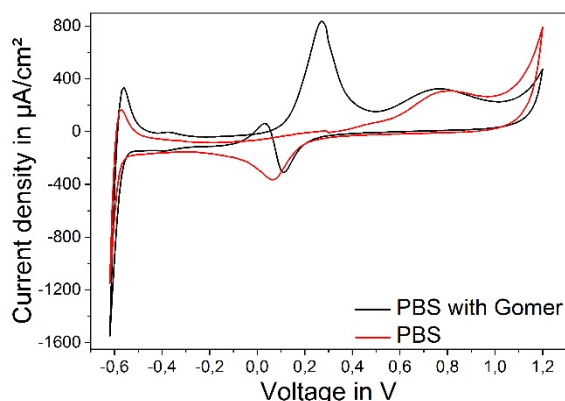


Figure 4: Cyclic voltammetric measurement of Gomer in PBS

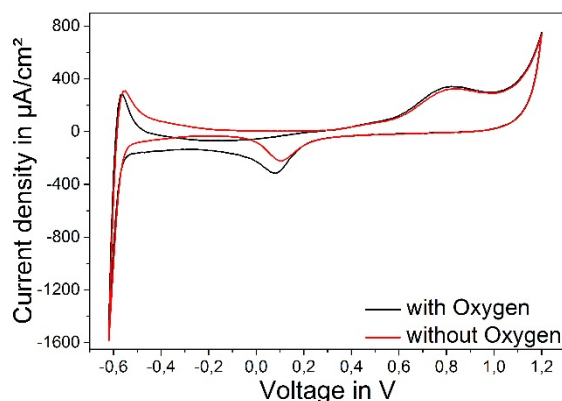


Figure 5: Cyclic voltammetric measurement of 70 μM PpIX in PBS with and without oxygen

3.2. Cell Measurements

T-47D breast cancer cells were successfully cultured on the microsensor system and incubated with the photosensitizer PpIX for PDT, see figure 3. The cellular oxygen concentration as indicator for cellular respiration was monitored before and after the therapy. The oxygen measurements were performed using a 3-step chronoamperometric protocol. The protocol runs with a waiting time of 50 s between each 10 s measurement. From every cycle the least 4 s are averaged to form a data point. In order to interpret the results of the cell measurements a calibration of the current density compared to the oxygen concentration was done. The chip was measured under cell environmental conditions at a temperature of 37 °C with oxygen concentrations between 0.5 and 20.95 %, which correspond to a range between 4.9 and 198 μM of dissolved oxygen. The resulting calibration curve of the oxygen sensor with a slope of $-0.7 \mu\text{A}/\text{cm}^2/\mu\text{M}$ is shown in figure 6. A linear dependency between current density and oxygen concentration was found. The electrochemical measurements revealed the increase in the oxygen concentration due to reduced cell respiration and cell death after the treatment, see figure 7. The

photodynamic dose was chosen to demonstrate an incomplete treatment. Approximately 10 h after the treatment a slow decrease in oxygen tension became visible. Here, it was possible to observe *in situ* the increase in respiration caused by cell repopulation. The sudden increase and subsequent decrease at approximately 65 h is caused by the medium exchange and the resulting disturbance of the oxygen diffusion gradient dropping towards the cells.

These results demonstrate the importance of continuous metabolic monitoring as phenomena like the repopulation effect would be overseen using single point measurements only.

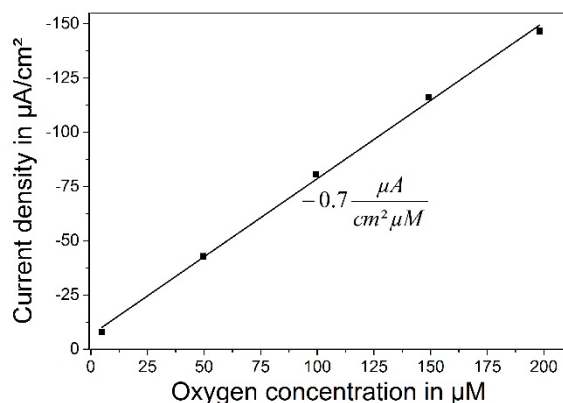


Figure 6: Oxygen sensor calibration in cell culture medium at 37 °C

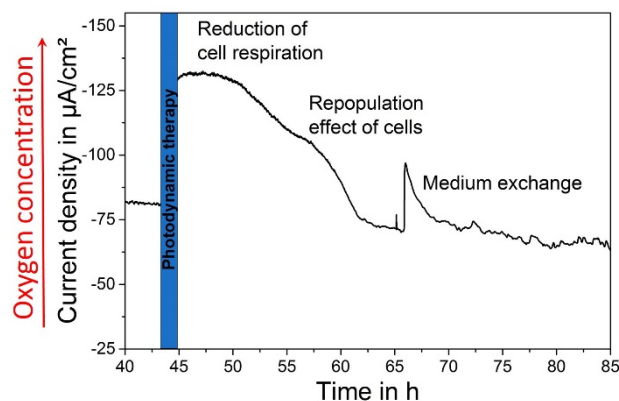


Figure 7: Pericellular oxygen measurement before and after photodynamic therapy on T-47D breast cancer cells

4. Conclusion and Outlook

The presented results show the applicability of the microsensors system to monitor oxygen concentration during PDT in a cell culture during and after treatment. It was demonstrated by the electrochemical characterization that the presence of the photosensitizer drug itself does not lead to any significant interference with sensor readings. A protocol for efficient culturing T-47D breast cancer cells on the sensor chip has been established in order to provide an on-chip treatment. The results suggests that the cellular respiration can be used as an indicator for cell death. Additionally, the system enables the monitoring of possible and undesired repopulation effects after the treatment. It became evident, that the continuous metabolic monitoring reveals effects, which would be overseen using single point measurements only.

Future work will focus on the combination of sensors for different metabolic parameter, in a first step by the integration of biosensors for glucose and lactate.

References

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