Chemical Sensors for Bio-Scientific Research – From *in Vitro* to *in Vivo*

J. Kieninger, K. Aravindalochanan, H. Flamm, A. Weltin, B. Enderle, G. A. Urban
Albert-Ludwigs-Universität Freiburg – IMTEK
Georges-Köhler-Allee 103, 79110 Freiburg, Germany

Chemical sensors provide important information about metabolic processes in both, *in vitro* (in cell culture) and *in vivo* (in living organisms) measurements. Parameters of interest are oxygen, pH, glucose and lactate. Depending on the objective of the experiments, nitric oxide, reactive oxygen species (superoxide) and glutamate are also important for biological measurements.

This paper will provide an overview about the similarities and differences between electrochemical sensor systems for *in vitro* and *in vivo* applications. Different types of cell culturing (adherent cells, suspension cultures) are discussed for *in vitro*. The various implications for measurements *in vivo*, as well as their different locations in the brain or muscle tissue, are illustrated.

1 Electrochemical sensors

The application of electrochemical sensors allows the measurement of several different analytes with a sensor array comprising electrodes with different functionalization. This is an essential advantage when comparing electrochemical and optical sensor methods with respect to multi-parameter monitoring application. Parallel measurements of several parameters, as well as with multiple sensor systems, can be easily achieved by electrical multiplexing.

1.1 Amperometric sensors

Amperometric sensors allow oxygen, nitric oxide, glucose, lactate, glutamate and superoxide measurements. The analyte or subsequent intermediate species is oxidized or reduced at the corresponding working electrode. While the *Clark-type* oxygen sensor [1, 2] is attractive due to the separation of the measurement medium and the sensor electrolyte by a gas-permeable membrane, this approach hinders the simple integration of such a sensor in a sensor array. Therefore chronoamperometric oxygen sensor protocols were developed to provide stable operation of a direct amperometric sensor in biological media without the need of a gas-permeable membrane [3, 4].

Biosensors for glucose, lactate or glutamate are based on the appropriate enzyme (glucose oxidase, lactate oxidase or glutamate oxidase), embedded in a hydrogel membrane on top of the sensing electrode. Hydrogen peroxide, as the subsequent intermediate species, is oxidized at the working electrode resulting in a sensor current proportional to the analyte concentration. Nitric oxide (NO) sensors are based on the oxidation of NO at a platinum or gold working electrode. Selectivity is achieved by an appropriate electrochemical detection technique, and due to application of semi-permeable membranes [5, 6].

1.2 Potentiometric sensors

The most important potentiometric sensors in biological applications is the pH sensor. In microtechnology, long-time stable pH sensors are usually based on a metal oxide as the indicator electrode. Iridium oxide [7] is mainly used for this purpose. Other potentiometric sensors of interest could be the potassium sensors or platinum electrodes for redox potential measurements.

1.3 Reference electrodes

Both, amperometric sensors operated in three-electrode mode, and potentiometric sensors require a stable reference electrode. The application of a silver/silver chloride electrode deposited onto the sensor chip by directly immersing it in the biological measurement medium is often sufficient. This is enabled by a rather constant chloride ion concentration around 0.1 M. In case of the cell culture medium RPMI 1640 (*in vitro*) it is 108 mM, in serum (*in vivo*) with the normal value in the range 97 – 108 mM [8]. If the

chloride ion concentration is suspected to change during the measurement duration or if the smallest traces of silver ions could harm the measurement environment, the integration of a liquid-junction based reference electrode is also possible with micro-sensors [8].

2 Sensor system for *in vitro* application

The *Sensing Cell Culture Flask (SCCF)* is a technical platform for *in vitro* research comprising a conventional tissue culture flask with integrated sensor chips (see figure 1). The sensor chips are based on glass substrates providing the possibility to use inverted microscopy for optical inspection of the cells (see figure 2). The main focus of the system integration was to provide a device which least disturbs the routine procedures applied during cell culturing. Equipped with different electrochemical sensors, the system can be used for long-time monitoring during cell culture experiments as well as to study short-time responses of cells to external stimuli. [8, 9, 10]

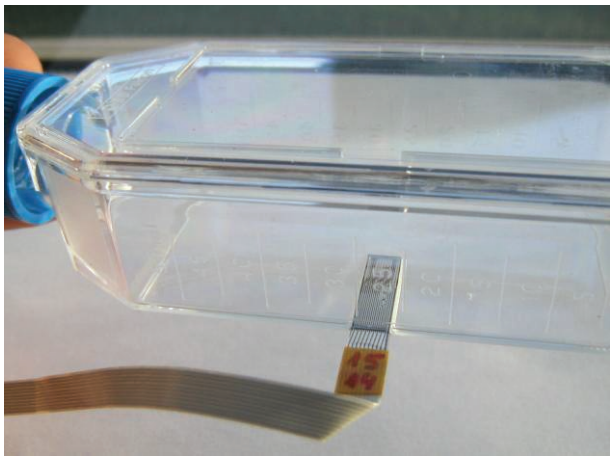

Figure 1: Sensing Cell Culture Flask (SCCF) allowing pericellular measurements in vitro*. The transparent sensor chip is embedded in a conventional tissue culture flask.*

Figure 2: T-47D breast cancer cells on a SCCF sensor chip. The transparent sensor chip allows optical inspection of the cell by inverted microcopy.

The most important parameter in *in vitro* is the oxygen concentration. The oxygen tension close to the cells (pericellular) can significantly deviate from what is preset in the incubator atmosphere. This occurs because of concentration gradients along the cell culture medium due to the cellular respiration. With the SCCF system equipped with oxygen sensors it was shown, that cells in normoxic condition (19.9% oxygen in incubator atmosphere) can reach pericellular oxygen levels as low as it would be expected in hypoxic incubation condition (4% oxygen). Those experiments illustrate the necessity of pericellular oxygen monitoring especially in the field of hypoxia (tumor) research. [10]

3 Sensor system for *in vivo* application

In contrast the *in vitro* application described above, the usage of sensor systems *in vivo* should avoid rigid substrates such as silicon or glass. A multilayer technology based on flexible polyimide films and isolation layers which can be applied by hot-roll lamination allowing the fabrication of flexible sensor strips. For multiparameter monitoring *in vivo* a flexible sensor strip was developed (see figure 3).

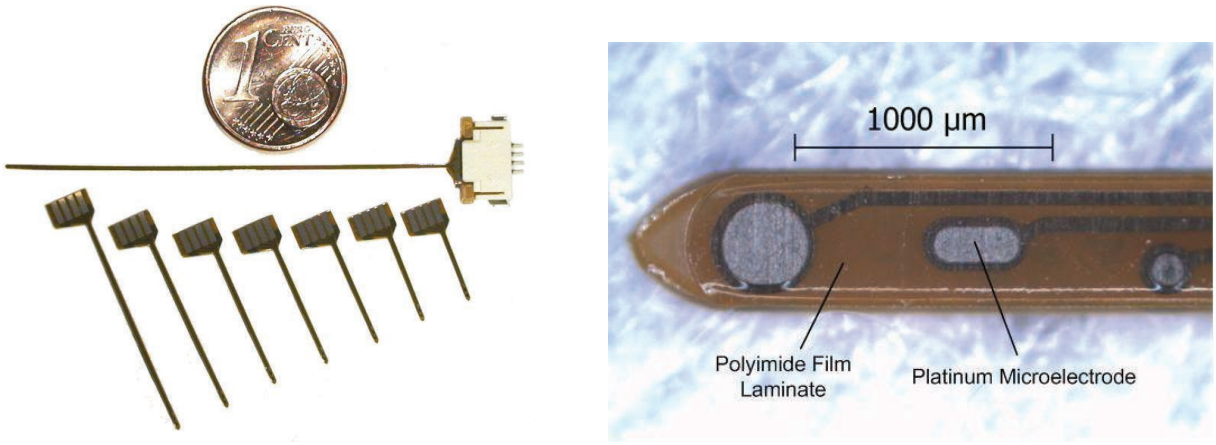

Figure 3: Flexible sensor strips for in vivo *application: The strips can be fabricated in different length (left), detail view of the tip comprising the sensor electrodes (right).*

The choice of the applied technology intended to obtain flexible sensors with enough stiffness to allow penetration into muscle or brain tissue (see figure 4). A balance has to be found between higher stiffness allowing precise insertion and the danger of tissue damage, irritation or inflammation. Results from measurements with oxygen and biosensors in neurological research as well as in muscle tissue demonstrate the challenges during animal experiments.

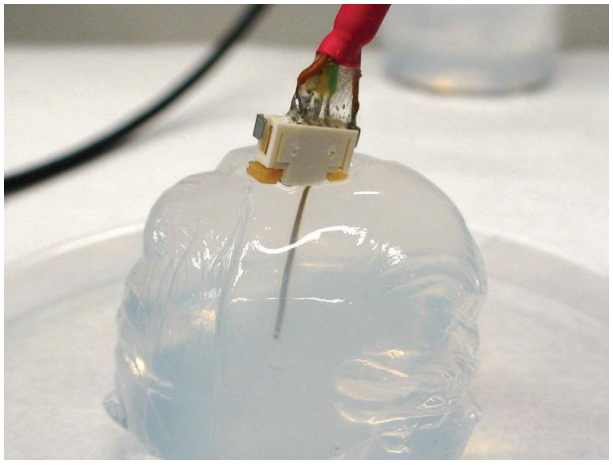

Figure 4: In vivo *sensor strip inserted in a gel simulating the properties of brain tissue. The flexible sensor strip shows enough stiffness to be inserted in muscle or brain tissue.*

4 Conclusion

It can be concluded that the demands for the individual electrochemical sensor points are comparable *in vitro* and *in vivo*, while aspects of system integration differs significantly. Transparent sensor chips are favored for the application of sensor arrays in cell culture experiments. On the other hand for *in vivo* sensors a technology resulting in flexible sensor strips is highly recommended.

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