# µRESPIROMETER TO DETERMINE THE OXYGEN CONSUMPTION RATE OF MAMMALIAN CELLS IN A MICROFLUIDIC CELL CULTURE

*Frank Bunge[1], Sander van den Driesche[1], Anya Waite[2], Ursula Mirastschijski[3] and Michael J. Vellekoop[1]*

[1]Institute for Microsensors, -actuators and –systems (IMSAS), University of Bremen, GERMANY

[2]Alfred-Wegener-Institute, Bremerhaven, GERMANY

[3]Dept. of Plastic, Reconstructive and Aesthetic Surgery, Klinikum Bremen-Mitte and, Centre for Biomolecular Interactions Bremen, University of Bremen, GERMANY

## ABSTRACT

We present a novel µrespirometer to determine the oxygen consumption rate (OCR) of mammalian cells. The oxygen concentration is measured with the fluorescent dye PtTFPP in a polystyrene-matrix. This film is integrated into a closed microfluidic chip made out of only oxygen-impermeable materials like glass and silicon. This results in a low drift and thus allows long-term measurements with living mammalian cells. With this chip, we determined the OCR of basophil RS-ATL8 cells as 32 amol/(cell·s).

## INTRODUCTION

Oxygen consumption is a key parameter to describe the metabolism, viability and physiological behavior of living cells. On one hand, these properties are influenced by the physical and chemical environment. Drug tests are a common example where the chemical environment is altered in order to influence the cell metabolism. This influence can be evaluated by measuring the oxygen consumption rate. On the other hand, the cell cultures need sufficient oxygen supply for good growth conditions. Consequently, knowledge about the required oxygen supply is needed for the proper design of new culturing devices like microfluidic chips.

Still, there is only little knowledge available about the oxygen consumption rates of mammalian cell cultures due to the lack of appropriate devices. Microfluidic chips are suited for such measurements, as the volume is small and available oxygen is consumed faster than in traditional cell cultures [1]. The oxygen concentration can be measured with Clark-electrodes inside such a microfluidic chip [2]. These electrodes are easy to integrate by standard clean room technologies but consume oxygen themselves, which influences the oxygen uptake of cells. Another method is the usage of an oxygen-sensitive fluorescent dye that is embedded in a matrix [3]. A commonly used matrix is polystyrene (PS) which adheres only poorly on smooth surfaces of glass or silicon wafers and does not withstand high temperatures of typical anodic bonding procedures. Therefore these films are usually combined with PDMS as chip material which is permeable to gases and thus impair long-term measurements.

The measurement in our device is also carried out with a fluorescent film of the Platinum(II)-5,10,15,20-tetrakis-(2,3,4,5,6-pentafluorphenyl)-porphyrin (PtTFPP), which is embedded in a polystyrene-(PS)-matrix because of the high photo- and chemical stability and good biocompatibility properties compared to other films [4]. The film is deposited inside a microfluidic chip made out of glass and silicon with adapted processes. Both materials are impermeable to oxygen which makes the chip suitable for long-term measurements. So by measuring the oxygen concentration, the oxygen consumption can be calculated with the known volume and number of cells.

## DESIGN AND FABRICATION

### Chip Fabrication

52 devices are fabricated at once out of a glass and a silicon wafer. The silicon layer contains the 200 µm deep microfluidic chambers which are fabricated by a DRIE-etching step. Subsequently, the inlet and outlet are etched as well with a DRIE-process from the backside of the wafer.

The glass wafer is roughened by powderblasting at the positions of the oxygen sensitive film in order to increase the adhesion of the film. The resulting rms-roughness is Rq=3.84 µm. Afterwards, the dry film resist Etertec E8015 is laminated and patterned on the wafer. Consequently, a cocktail of 10 ml toluol, 500 mg PS and 10 mg PtTFPP is deposited and placed in vacuum in order to form the PS-PtTFPP-film by evaporation of the toluol. After 1 hour vacuum treatment, the film is patterned by a lift-off-process of the dry-film-resist during two hours in a $CaCO_3$-solution. The film decreases the roughness to Rq=1.01 µm. Finally, the glass-wafer is bonded anodically to silicon. As polystyrene is only stable up to 240°C, the bonding process is performed in vacuum at 230°C, 1000 V and 2.6 bar pressure for 1 hour. The entire process is summarized in Figure 1.

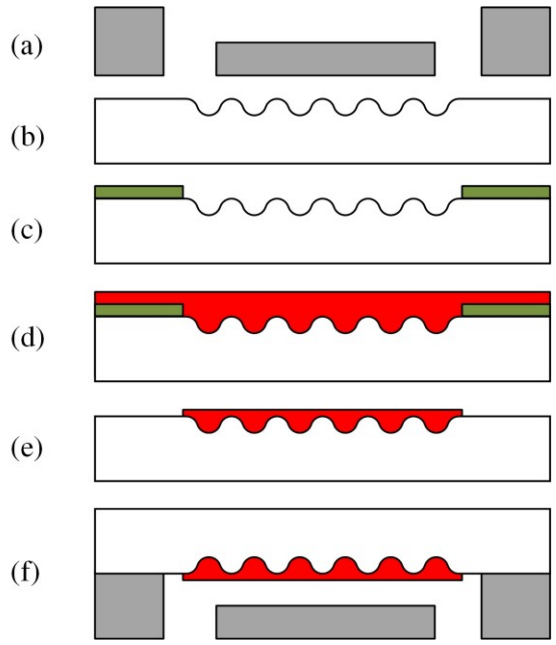


*Figure 1: Fabrication of the device: (a) 380 µm thick silicon containing 200 µm deep, DRIE-etched channels and inlets (b) glass wafer with partially roughened surface by sandblasting (c) patterning dry film resist Etertec E8015 (green) (d) deposition of sensor film (red) (e) lift-off by removing the dry-film-resist (f) anodic bonding of both wafers*

The length of the microfluidic chamber is chosen as 16.8 mm to minimize the influence of oxygen diffusion through the inlets and outlets into the device. The lift-off process has a high success rate in case of large structures but still shows some small imperfections on the boundaries of the film. Therefore the widths of the chamber and of the film are 2.5 mm and 2.1 mm respectively. The small gap between the film and the silicon wall can be used to observe the cells. The final chip with the outer dimensions of 11.5 x 9.5 x 0.9 mm³ is presented in Figure 2.

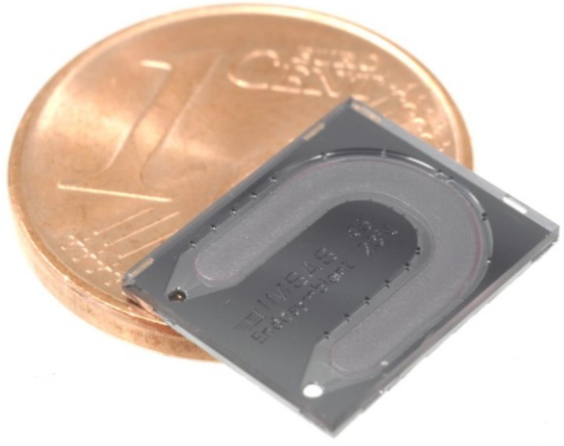

*Figure 2: The chip after the fabrication process*

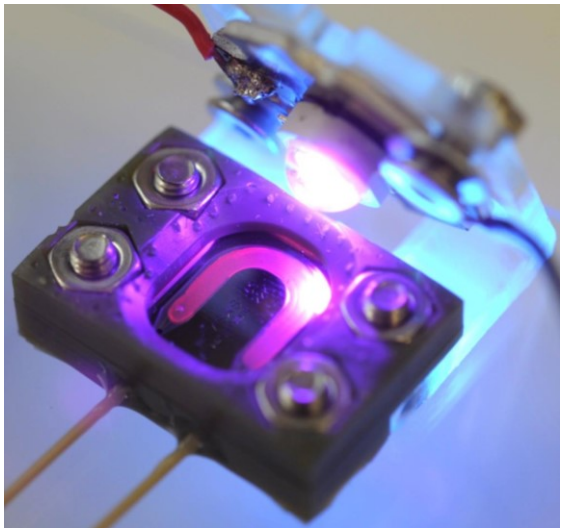

*Figure 3: The setup of the chip in the housing and the LED*

**Assembly of the µrespirometer**

The fluorescent film inside the chip requires UV-excitation. An UV-LED with a wavelength between 395nm and 410nm is used, which was purchased from LED-TECH.DE optoelectronics GmbH. A holder for the LED is fabricated by 3D-printing to mount the LED and the chip in a reproducible arrangement (see Figure 3). The mean distance between the LED and the film is 11 mm and the angle of incidence is fixed to 54°. So the LED is still very close to the chip enabling high light intensity but sufficiently far away to observe the assembly under a microscope.

The chip has to be connected to the macroscopic world. Therefore, the holder is also used to connect the µrespirometer to capillary tubes. The connection between the chip and the holder is sealed with non-cytoxic O-rings from Parker Hannifin GmbH made out of ethylene propylene-diene monomer rubber (EPDM).

The film emits light at 650 nm which is filtered with a long-pass filter with a cut-off frequency of 590 nm. The emitted light is observed through a microscope and recorded with a camera. The intensity is evaluated afterwards as the mean value of 0.25 mm² big squares of the film. As the film and the recorded image are larger, several spots are measured and analyzed simultaneously.

## EXPERIMENTAL RESULTS

**Characterization of the system**

The performance of the µrespirometer was evaluated with water at different oxygen-concentrations. These concentrations were measured with the commercial oxygen sensor FireStingO2 of PyroSciences GmbH before inserting the samples into the chip. The relative intensity was determined once at five predefined spots and a second time at the same spots after refilling the chamber. The measurements as shown in Figure 4 confirm the good

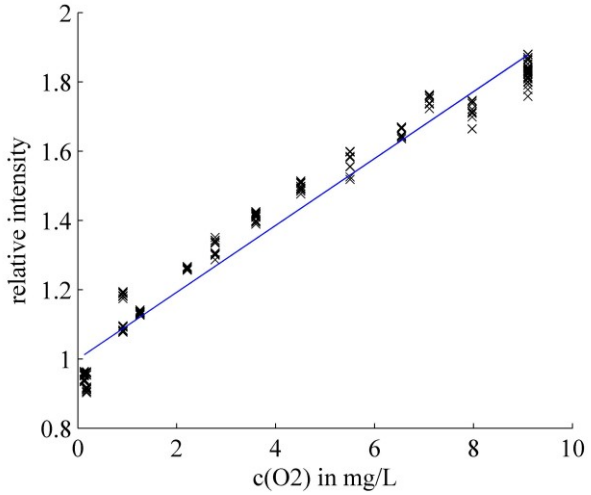


*Figure 4: Relative intensity at different concentrations for each 10 measurements and a fitted linear curve ($R^2$ =0.97)*

linearity as described by the Stern-Volmer equation:

$$\frac{I}{I_0} = 1 + Kc_{O_2}, \qquad (1)$$

where $I$ is measured intensity, $I_0$ the intensity without oxygen, $c_{O_2}$ the concentration of dissolved oxygen and the constant $K$. With the measured date, $K$ is determined as $K = 0.096 \pm 0.002 \frac{l}{mg}$ with an overall coefficient of determination of $R^2$ =0.97.

The response time of the sensor is measured as 0.88±0.03 s for a decrease of the concentration from 9.3 mg/l (i.e. fully $O_2$ - saturated water with partial oxygen pressure of 0.2 atm) to 0 mg/l and 0.93±0.02 s in the reverse direction. So the sensor reacts much faster than the expected oxygen consumption of cells which is in the range of minutes to hours.

A long-term measurement of deoxygenated water shows that the measured concentration increases from 0 mg/l to 0.39±0.09 mg/l within the first hour as shown in Figure 5. Afterwards, there measured concentration increased only by 0.0117±0.0005 mg/l/h which shows the good long term stability compared to the oxygen concentration of fully air saturated water of 9.1 mg/l.

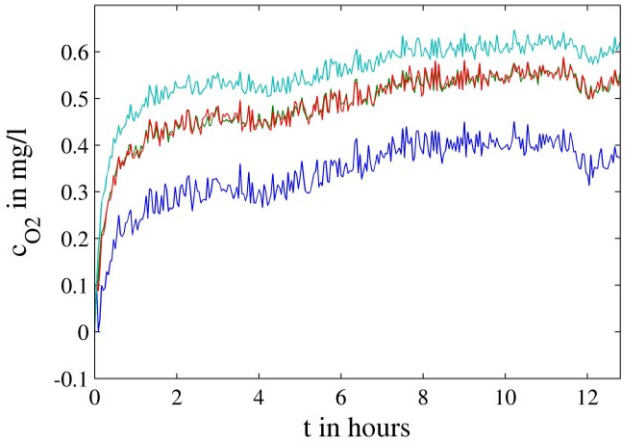


*Figure 5: Long-term measurement with deoxygenated water at 4 different spots. Fully oxygenated water has a concentration of 9.3mg/l*

**Measurement of the OCR of basophilic cells**

To verify the performance of the respirometer, we inserted basophilic cells RS-ATL8 with a concentration of 3.3E6 cells/ml into the device (see Figure 6). The initial oxygen concentration was measured as 8.6 mg/l.

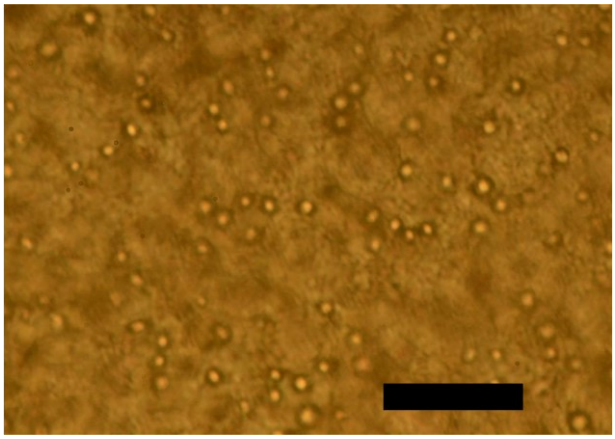

*Figure 6: Basophilic RS-ATL8 cells under the sensing film after inserting into the chip. The scale bar is 100 µm*

Within 42 min, the concentration decreased linearly to 0 mg/l (see Fig. 7). So the oxygen consumption rate is calculated as 32 amol/(cell·s). To our knowledge, there is no other information published about the oxygen uptake of these cells. However, the determined OCR is similar to published rates of other cell types [5].

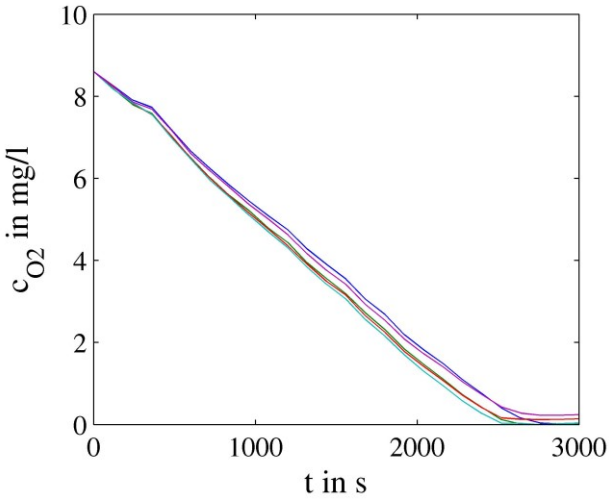


*Figure 7: Oxygen concentration of a cell culture of RS-ATL8 cells with a concentration of 3.3E6 cells/ml*

## CONCLUSION

We present a µrespirometer as a device to determine the oxygen consumption rate of living cells. The oxygen uptake is evaluated by measuring the oxygen concentration in the cell culture media with a known volume and cell density. The measurement requires that only biocompatible materials are used and the only change of the oxygen concentration is due to respiration of mammalian cells. Therefore, the measurement principle should not consume any oxygen and the device has to be sealed to avoid oxygen diffusion through the material.

Our device fulfills these requirements by using a fluorescent film of PtFTPP which is embedded in a polystyrene matrix. The intensity of the fluorescence depends mainly on the oxygen concentration but does not consume any oxygen. The microfluidic chip, in which this film is embedded, is made out of biocompatible but oxygen-impermeable materials like glass and silicon. The film is deposited on partially roughened glass wafers to achieve good adhesion properties and structured with a lift-off-process. The sealing is realized by anodic bonding of the glass and silicon at very low temperatures in order to avoid damages of the polystyrene film.

The deposited film is relatively thin, which results in the fast response time of less than 1 s. Additionally, the integration of the film in a sealed device allows a very low drift. The combination of both properties enable the usage of the presented µrespirometer for short-time measurements with high OCRs or high cell density but also long-time measurements with low OCR and a low cell density.

The determined oxygen consumption rate of basophilic RS-ATL8 cells (density is 3.3E6 cells/ml) is constant over time and equals 32 amol/(cell·s).

In conclusion, the presented µrespirometer contains fluorescent dye in a polystyrene-matrix which is embedded into a microfluidic chip made out of gas-impermeable materials. This integration results in a fast response time but also good long-term stability. Furthermore, only biocompatible, non-cytotoxic materials are used for the setup. Consequently, this chip is well suited for the measurement of the oxygen consumption rate of mammalian cells.

## ACKNOWLEDGEMENTS

We would like to thank Gazanfer Belge, Radhika Dhekane, Sorge Kelm, Karen Perry, Arlo Radtke and Mario Waespy for thoughtful discussions. The authors also would like to thank Dr. Nakamura from the National Institute of Health Sciences, Tokyo, Japan for providing the RS-ATL8 cell line.
This work is a part of the research project "LifeChip" which is supported by the Federal Government's and Federal States' Excellence Initiative in the framework of the institutional strategy of University of Bremen, Germany.

## REFERENCES

[1] D. Papkovsky, R. Dmitriev, "Biological detection by optical oxygen sensing", *Chem. Soc. Rev.*, 2013, 42, 8700-8732
[2] P. Oomen, M. Skolimowski, E. Verpoorte, "Implementing oxygen control in chip-based cell and tissue culture systems", *LabChip,* 2016, 16, 3394-3414
[3] B. Ungerböck, V. Charwat, P. Ertl, T. Mayr, "Microfluidic oxygen imaging using integrated optical sensor layers and a color camera", *LabChip*, 2013, 13, 1593-1601
[4] X. Wang and O. Wolfbeis, "Optical methods for sensing and imaging oxygen: materials, spectroscopies and applications", *Chem. Soc. Rev.*, 2014, 43, 3666-3761
[5] B. Wagner, S. Venkataraman, G. Buettner, "The rate of oxygen utilization by cells", *Free Radic. Biol. Med.*, 2011, 51, 700-712

## CONTACT

*F. Bunge, tel: +49 421 218 62579; fbunge@imsas.uni-bremen.de