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Optochemical sensor instrumentation for in-vivo continuous monitoring of adipose tissue oxygen tension by microdialysis in humans

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Abstract

The continuous monitoring of adipose tissue oxygen tension (pO_2) in patients affected by obesity and type 2 diabetes is of high importance. Adipose tissue oxygen tension may play a decisive role in the development of insulin resistance from the patient. For this purpose is optochemical sensor technology combined with microdialysis particularly suitable for the development of minimally invasive instrumentation for medical application. We report about microdialysis-based oxygen optochemical sensor system based on the principle of the phase modulation fluorometry. An oxygen sensitive membrane is inserted in a miniaturised flow through cell (volume app. 5µl) and is interrogated by means of a fiber optic instrumentation integrated with the processing electronic unit. The interstitial fluid is extracted from the adipose tissue by means of a commercially available microdialysis catheter. In order to prevent the ambient oxygen diffusion using commercially available microdialysis catheter, the outlet of the catheter was replaced by steel tubing. The biological fluid is afterwards pumped at a flow rate of 2 µl/min into the flow through cell with the oxygen sensitive membrane inserted. The latter emits a luminescence signal dependent on the dissolved oxygen tension in the interstitial fluid.
This sensor instrumentation has been extensively validated both in laboratory and in clinical tests. It shows a resolution better than 1 mmHg and accuracy better than ±1 mmHg over the whole measurement range of 0 – 300 mmHg. The response time including the flow time of the interstitial fluid from the catheter to the sensitive membranes is of about 10 min.

Keywords: Optochemical sensor instrumentation, adipose tissue oxygen tension, Microdialysis, In-vivo tests

Introduction

The continuous monitoring of adipose tissue oxygen tension (pO_2) is an important indicator of the health conditions of living organisms and can be valuable in determining the physiologic status of critically ill patients. In particular, adipose tissue dysfunction may lead to chronic inflammation that predisposes to type 2 diabetes. Recent cell culture and preclinical experiments suggest that this type of dysfunction can be explained by low levels of oxygen associated with obesity [1].
Although many sensors for the measurement of gas levels in the biomedical field have been already developed, the only commercially available for the monitoring of adipose tissue pO_2, are needle-type sensors. Those suffer of the typical drawbacks of a point measurement, which is strongly dependent on the position of the sensor tip inside the adipose tissue and show high fragility due to the small dimension of the sensor heads.
The combination of an optical sensing approach with a microdialysis technique appears to be more appropriate for studying metabolism in tissues [2, 3]. In this method a perfusion solution is swept through a hollow fibre membrane exposed to the sample medium. Low molecular weight substances in the interstitial fluid surrounding an implanted microdialysis catheter are allowed to diffuse across the membrane and to equilibrate with the perfusion solution. An important advantage of this system is that adipose tissue pO_2 is measured over a much larger area compared to commercially available needle-type oxygen sensors
The combination of an optical sensing approach with a microdialysis technique has been already used for monitoring of dissolved gases and pH in a flow loop [4, 5]. Pasic et al. have described a microdialysis-based glucose-sensing system with an integrated fibre-optic hybrid sensor [6].
We have reported about an optochemical measurement system for continuous monitoring of adipose tissue oxygen tension in biological fluid using a miniaturized flow-through cell that is coupled with a microdialysis catheter [7, 8]. The adopted sensing scheme was phase fluorometry, which is based on the dynamic quenching due to oxygen of the photo-excited luminophore Platin(II) meso-tetra(pentafluorophenyl) porphyrin (Pt-TFPP). The response

characteristics of the optical oxygen sensor have been fully characterised both in laboratory and in in-vivo experiments on animals and healthy volunteers.
In the present paper, we present an improved microdialysis-based oxygen-sensing instrumentation with integrated miniaturized planar sensitive membrane for the continuous monitoring of adipose tissue oxygen tension. This instrumentation is suitable for measurement at a flow rate as low as 2 µl/min with a response time including the flow time from the catheter to the sensitive membrane of about 10 min. Parallel the production technology of the oxygen sensitive membranes has been also improved, so that it has been possible to produce altogether 60 sensitive membranes (with an oxygen sensitive spot of 2 mm of diameter), which allow the pO_2 measurement in the range between 0 and 300 mmHg with accuracy better than ±1 mmHg and resolution better than 1 mmHg. This instrumentation has been used to monitor adipose tissue oxygen tension in lean and obese humans.

Experimental

Chemicals and materials

The luminophore Platin(II) meso-tetra(pentafluorophenyl) porphyrin (Pt-TFPP) was obtained from Frontier Scientific Porphyrin Products. Polystyrene and chloroform were obtained from Aldrich. As perfusion solution Ringer solution was used, which is a water solution containing sodium chloride (NaCl), potassium chloride (KCl), sodium hydrogen carbonate ($NaHCO_3$) and calcium chloride ($CaCl_2$) in the same concentrations as in physiological fluids. All chemicals were reagent grade and were used without further purification. Tygon tubing (diameters 0.25/2.0 mm) used for the peristaltic pump were from Cole Parmer, Amex, USA. Steel tubing (diameters 0.15/0.30 mm) was from Rometsch GmbH, Germany. The high purity gases nitrogen 5.0 and oxygen 4.5 were purchased from Air Liquid, Austria.

Preparation of the oxygen sensitive layer

The sensor cocktail was prepared by dissolving Pt(II)-TFPP (4 w. % /g solid polystyrene) in chloroform. Afterwards the sensor cocktail was spread onto a transparent substrate; either a polyethylene terephthalate foils PET (Mylar foil with thickness 100 µm) or PET foils (50 µm thickness) coated with Al_2O_3. Knife coating and screen printing techniques have been used for production. After coating, the oxygen sensitive membranes have been dried at 60 °C for eight hours. In case of knife coating a successive mechanical reduction of the sensitive spot to a diameter of 2 mm has been applied. This procedure was not necessary in case of the screen printed sensitive membranes.

Optoelectronic interrogation unit

The fibre optic measurement system consists of an optoelectronic read-out module for measurements with optical fibres (core diameter 1000 µm) and of a miniaturised flow-through cell (volume app. 5 µl), where a planar oxygen sensitive membrane (diameter of the sensitive spot 2 mm) is allocated. This flow through cell is equipped with inlet tubing of inner diameter of 0.15 mm and outer diameter of 0.30 mm (Rometch GmbH). The outlet tubing and the flow chamber have an inner diameter of 2.0 mm. The outer diameter of the outlet tubing is 3.0 mm (see Figure 1). The inner diameter of the inlet was chosen to be 0.15 mm in order to match the inner diameter of the inlet of the microfluidic catheter. This geometry has been chosen to reduce the risk of overpressure during measurement, which would cause an overestimation of the dissolved pO_2.The length of the inlet is 20 mm whereas the outlet is 32 mm long.

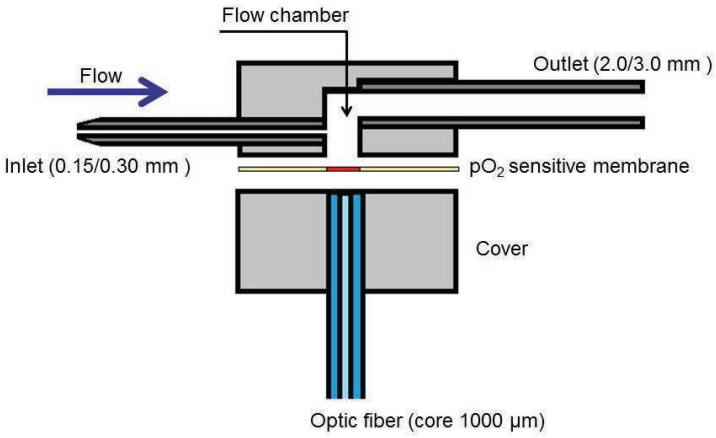

Figure 1. Schematic draw of the flow through cell

The optic set-up is integrated in the processing electronic unit and is based on a collimating optics, which converts the light of the excitation source (a Nichia NSPE590S LED) into a parallel beam. This is then focused by a GRIN lens into

an optic fibre, which guides the excitation light to the oxygen sensitive layer inside the flow-through cell. The luminescence collected by the same optic fibre is deflected by a dichroic filter at 45 ° (DC Red, Qioptiq Photonics GmbH & Co. KG) towards a silicon photodiode (Hamamatsu S5106). A set of optical filters (SCHOTT BG39, thickness 2 mm, for the excitation source and SCHOTT RG630, thickness 2 mm, for the photodiode) is used to separate the luminescence signal from the excitation light and other optical background signals (e.g. luminescence emitted by materials close to the O_2 sensitive membrane). A reference light source (Nichia NSPE590S LED) is used to compensate the reading of the measuring unit for changes of the electronic parameters of the components with fluctuations of the operating conditions (e.g. forward current through the LED and temperature). The main advantage of using fiber optic technology is the possibility to separate the optoelectronic instrumentation from the flow-through cell, thus allowing an extreme miniaturization of the cell itself. The new flow-through cell has size of only 20x13x4.5 mm, which makes it more flexible for use in in-vivo experiments and field tests in hospitals. A holder for the flow-through cell was also realized and mounted on a belt for an easy and fixed positioning on the abdomen of the subject during in-vivo experiment, as it is shown in Figure 2.

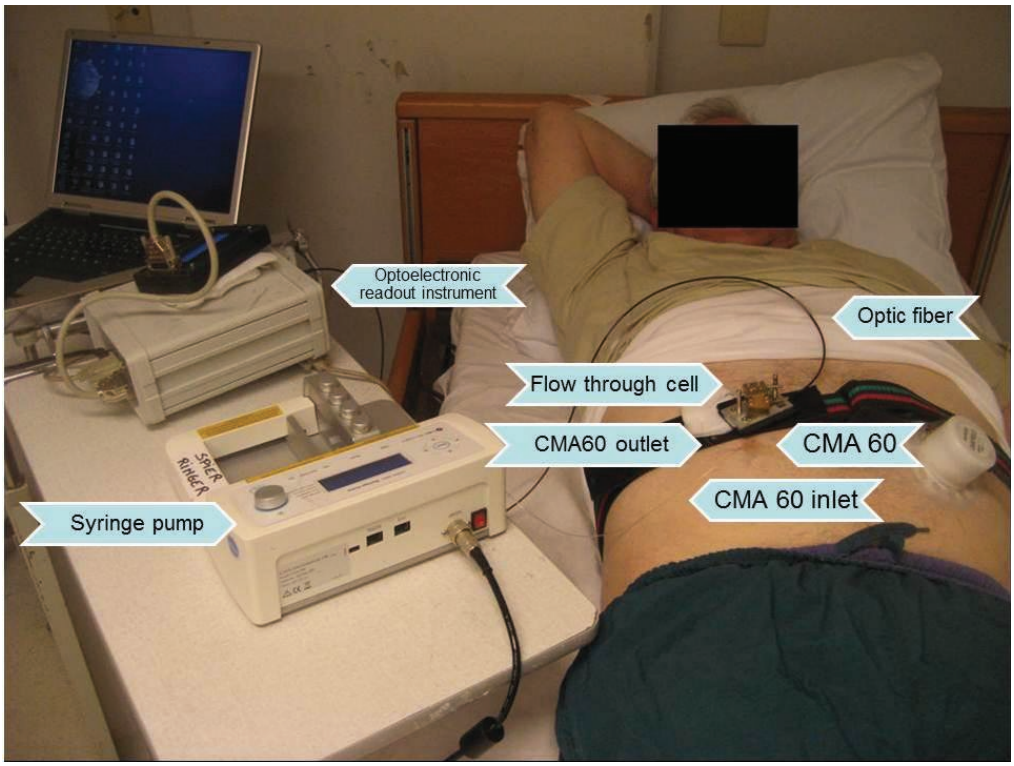

Figure 2. Use of the optochemical oxygen sensor instrumentation during a typical in-vivo test. The main components are the flow through cell, which is fixed near the umbilicus of the volunteer close to the position of insertion of the microdialysis catheter CMA 60. The luminescence emitted by the oxygen sensitive membrane is collected by an optic fibre cable and guided to the optoelectronic readout unit. The perfusion solution is swept into the microdialysis catheter by means of a syringe pump.

The outlet tubing of catheter was first cut as close as possible to the microdialysis membrane (at a distance of about 2 mm) and then connected with the flow through cell by means of steel tubing (diameters 0.15/0.30 mm). The length of the steel tubing was standardised at 90 mm in each experiment. In this way the ambient oxygen diffusion through the permeable tubing materials of the catheter can be neglected. This has been proven in oxygen recovery rate measurements, which have been carried out in laboratory. These measurements have been already exhaustively described in previously reported work [8]. The standardization of the length of all the connection tubing allows that the flow time from the catheter to the sensitive membrane is in all experiments the same.

Results and discussion

Laboratory characterization

All together 40 membranes have been produced by knife coating technique onto PET substrate and 20 membranes by means of screen printing onto PET substrate coated with Al_2O_3 as oxygen barrier.
All sensor membranes have been characterized in Ringer solution at the constant temperature of 37 °C. The temperature regulation was realised by using a water bath thermostat (Lauda RC 6 CP). The measurement medium has been equilibrated with a gas mixture of nitrogen and oxygen at defined concentrations (typically 0, 5, 10 and 21 % O_2) using a mass flow controller (Bronkhorst, The Netherlands). As reference instrumentation a paramagnetic gas purity analyser (Servomex, U.K.) was used. The solution has been then pumped by means of a peristaltic pump into miniaturised flow-through cell at the flow rate of 2.0 µl/min (Minipuls 3, Gilson, Germany). One example of the experimental protocol used for the laboratory characterisation of the planar sensitive membrane is shown in Figure 3.

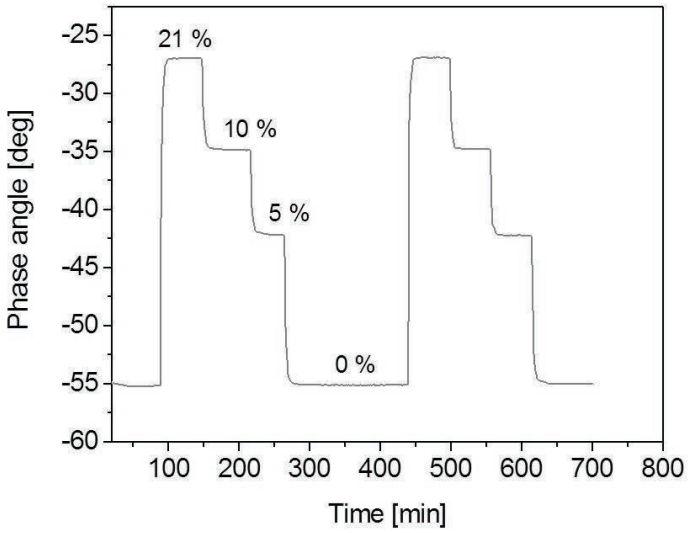

Figure 3: Example of the experimental protocol used for the characterisation of the miniaturised planar O_2 sensitive membrane. The measurement is carried out in Ringer solution equilibrated at defined O_2 concentrations (0, 5, 10 and 21 % O_2) at the constant temperature of 37 °C.

The first complete cycle of dissolved O_2 has been used for sensor calibration purpose, whereas the following cycles are used to check the specifications of the oxygen measurement (Table 1).

Table 1: Achieved specifications of the O_2 measurement with planar sensitive membranes compared to the optimum requirements defined in the project

	Specifications
Working range	0-300 mmHg
Resolution	1 mmHg
Accuracy	< 7% or ±1 mmHg

Both types of membranes perform satisfactorily with the same measurement specifications.

Delay time

The time needed for the extracted interstitial fluid to reach the sensitive oxygen membrane in the flow-through cell is indicated as delay time. This delay time is defined as the time required for the sensor output to change from its previous state to a final settled value within a tolerance band of the new value. It is a common practice to measure the delay time as the time needed for the sensor to reach the 90 % of the final output. This is usually indicated as the t_{90} of the sensor. Figure 4 shows the delay time t_{90} for an oxygen change from air saturated solution (circa 21 % O_2) to oxygen free solution (about 0 %).

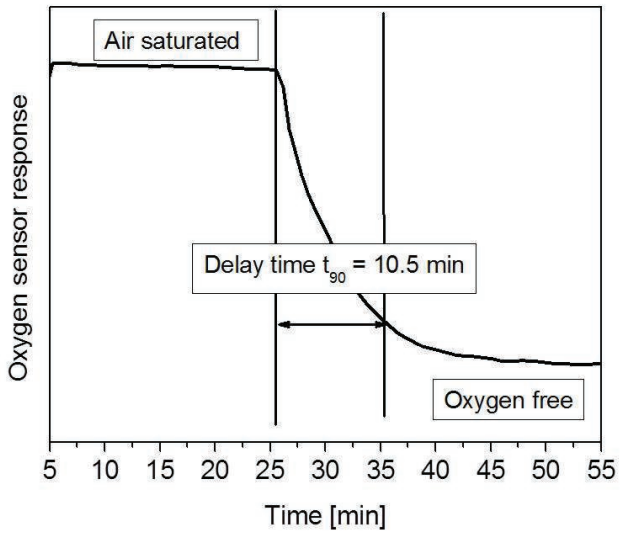

Figure 4: Measured delay time between 21 and 0 % of O_2 in case of one oxygen sensitive membrane

To perform this measurement, two different glass vessels filled with Ringer solution has been equilibrated with air and nitrogen. The catheter CMA 60 connected by steel tubing to the flow through cell has been first immersed in the vessel containing air saturated Ringer solution and the peristaltic pump has been started at the flow rate of 2 µl/min. After a stable value of the oxygen sensor instrumentation has been achieved, the pump has been shortly stopped to change the catheter in the oxygen free Ringer solution and the flow started again. The time needed to achieve the 90% of the final value as been recorded. Six different membranes of both types have been investigated and the averaged t_{90} could be estimated as 11.7 ± 4.6 min. Even if, the two different types of sensitive membranes (based on uncoated or Al_2O_3 coated PET substrate) perform satisfactorily with regard to the measurement specifications, it could be observed that, in single membranes based on the uncoated PET substrate, the response time increased above the specified time of 10 min, due to ambient oxygen diffusion through microscopic damages of the coated surface, caused by the mechanical reduction of the sensitive spot.

Clinical validation in humans

Validation protocol

To validate the sensor instrumentation in vivo in humans, a healthy, normal-weight male subject participated in an experiment carried out at Maastricht University Medical Centre (MUMC) in Netherlands under supervision of Dr. G. H. Goossens. The subject was asked to refrain from drinking alcohol and to perform no strenuous exercise for a period of 48 hours before the in-vivo experiment. On arrival, a microdialysis catheter (CMA 60, CMA microdialysis AB, Stockholm, Sweden) was inserted in the abdominal subcutaneous adipose tissue 6 cm right from the umbilicus after the skin was anesthetized by a lidocaine/prilocaine cream (EMLA, AstraZeneca BV, Zoetermeer, The Netherlands). After insertion, the probe was perfused with Ringer solution (Baxter BV, Utrecht, The Netherlands), supplemented with 50 mM ethanol, at a flow rate of 2.0 µl/min (CMA400 micro infusion pump, CMA microdialysis AB, Stockholm, Sweden).
After insertion, the system was allowed to equilibrate. After pO_2 had reached stable values, the probe was consecutively perfused with a vasoconstrictor and vasodilator, each for 60 min. There was a wash-out period of 120 min (Ringer infusion) before the infusion of the vasodilator was started.

Results and discussion of in-vivo experiments

A stable baseline pO_2 of 52.5 mmHg was reached (time in Figure 5 from -30 to 0 min). As expected, local administration of the vasoconstrictor in adipose tissue (t0-60 min) decreased both adipose tissue blood flow and adipose tissue pO_2 (37.3 mmHg, Figure 5). During the wash-out period (t60-180min), pO_2 values returned to baseline again (53.5 mmHg). Subsequently, local infusion of the vasodilator increased both adipose tissue blood flow and pO_2 values (t180-240min) (74.8 mmHg, Figure 5). This experiment clearly shows that pO_2 values can be reliably measured in-vivo in humans, and can be modified by changes in tissue blood flow.

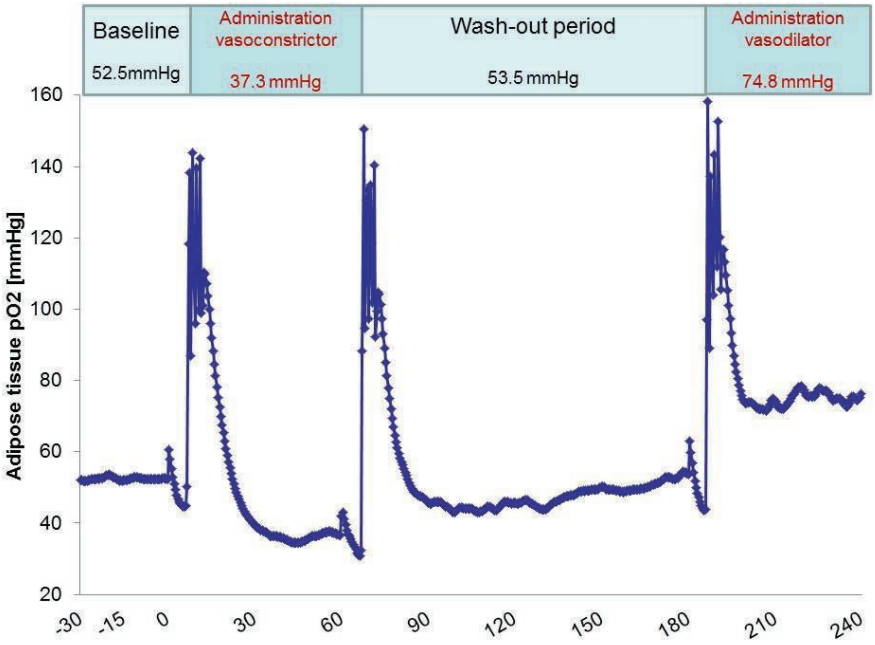

Figure 5: Measured abdominal adipose tissue pO_2 with optochemical sensitive membrane under stable baseline conditions (local Ringer administration up to time 0), during local administration (2 µl/min) of a vasoconstrictor between 0 and 60 min, followed by wash-out period with Ringer infusion (from 60 to 180 min). At 180 min an infusion of a vasodilator was started up to 240 min. The experiment was carried out at Maastricht University Medical Centre (MUMC) in Netherlands under supervision of Dr. G. H. Goossens.

The rapid increase of the measured pO_2 at the beginning of each drug administration is caused by ambient oxygen, which has been introduced in the fluidic during the change of the infusion solution.
After the in-vivo experiments few sensitive membranes were calibrated again in laboratory and the deviations of the pO_2 values obtained by the new calibration have been calculated and plotted against the actual pO_2 values (Figure 6). It can be clearly seen, that the change in the calibration of the sensitive membrane after the in-vivo experiment causes very small discrepancy from the actual pO_2 values. This discrepancy can be estimated in less than $\pm$ 0.5 mmHg over the whole range of measurement. This result is very excellent considering that the oxygen sensitive membranes have been recalibrated about one year after the first laboratory calibration and after in vivo experiments of a typical duration of 5 – 6 hours.

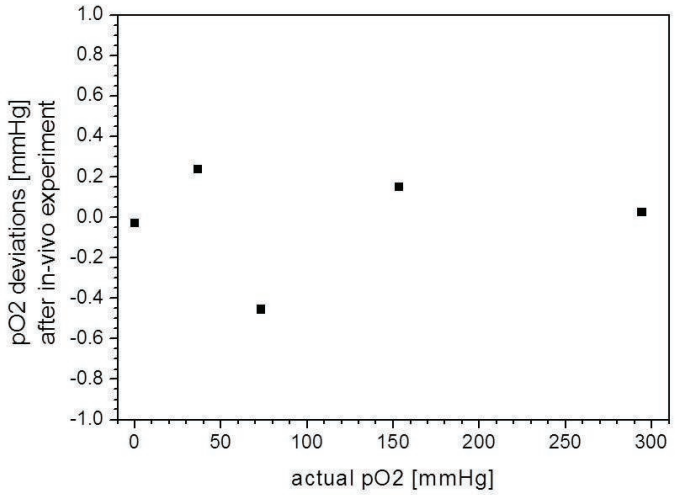

Figure 6. Deviations of the pO_2 values measured for single oxygen sensitive membrane after the in vivo experiment compared to the calibration values.

Conclusion

We report about a microdialysis-based oxygen-sensing instrumentation with integrated miniaturized sensitive membrane for the continuous monitoring of adipose tissue oxygen tension. In comparison to previously reported instrumentation [8], this is suitable for measurement at a flow rate as low as 2 µl/min with a response time including the flow time from the catheter to the sensitive membrane of about 10 min. The production technology of the oxygen sensitive membranes has been also improved, so that it has been possible to produce altogether 60 sensitive membranes, allowing the pO_2 measurement in the range between 0 and 300 mmHg with accuracy better than ±1 mmHg and resolution better than 1 mmHg. This instrumentation has been validated in in-vivo experiments, in which the adipose tissue oxygen tension in humans has been monitored under rest conditions and after the local administration of vasoconstrictor and vasodilator. The experiments were carried out at Maastricht University Medical Centre (MUMC) in Netherlands under supervision of Dr. G. H. Goossens. The agreement between the observed pO_2 adipose tissue tension and the expected behavior due to the induced alteration of the adipose tissue blood flow allows concluding that the system is suitable for in vivo monitoring of pO_2 associated with adipose tissue dysfunction.

After in-vivo experiments few sensitive membranes were calibrated again in laboratory and deviations smaller than $\pm$ 0.5 mmHg have been observed. This is evidence that the membranes have an excellent storage and operating stability and that contact to biological fluid does not affect the response of optochemical measurement instrumentation.

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