Cell culture pO_2 monitoring with a fiber optic oxygen sensor

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ABSTRACT

Monitoring of cellular cultures oxygen partial pressure (pO_2) in liquid phase with a fluorescence quenching based fibre optic oxygen sensor is reported. The sensitivity of the fibre optic oxygen sensor was evaluated in gas phase and calibrated in liquid phase before the monitoring of cell culture pO2 with a luminescence phase detection system. In the experiment of cell culture pO_2 monitoring the porcine kidney (PK-15) cells were used as the cell samples and the experimental results showed that the sensor is suitable for monitoring of pO_2 in gas and liquid phase. And we found there are different measured pO_2 level in liquid phase for different cell densities in cell culture at ambient air environment, which has a PO_2 of 21kPa corresponding to an oxygen concentration of 21%. Under the condition of low pO_2 in gas phase and high cell density, the cell metabolism could shift pO_2 in the liquid phase much lower than that would be from the O_2 gas/air equilibrium coefficient, which indicates that the cellular oxygen uptake rate exceeds the oxygen diffusion rate. Therefore there is a need for direct pO_2 monitoring in cell culture, particularly in static high density cell culture systems, in which oxygen mass transfer is limited.

Keywords: oxygen partial pressure (pO_2), fiber optic oxygen sensor, porcine kidney cells, cell culture

1. Introduction

An incubator with temperature/humidity/CO_2 control and simple plastic disposable flasks filled with sterile media are employed for cell culture. The most cultures are worked under static ambient air conditions, where the flasks are simply placed in the incubator. Cell survival, proliferation, and differentiation are regulated by the extracellular pO_2 and the oxygen concentration in the culture medium was simply the result of having the medium equilibrate with ambient air. With no cells in the culture liquid medium, oxygen concentration of 21% in gas phase gives a pO_2 of 21kPa in liquid phase; With cells in the culture liquid, the actual pO_2 in close proximity to the cells is dependent on two facts: oxygen supply to the cells by diffusion through the media/flask walls and oxygen consumption by the cells. Normally, the pO_2 in liquid phase is unmonitored and uncontrolled in most cell cultures. This was because of a first approximation that the

cells are provided with ‘‘enough’’ oxygen. However, it now has firmly established in vivo that most cells, such as stem cells, are in environments with low or very low PO_2 level (Simon and Keith 2008; Lin et al., 2008). In addition, it has been shown in vitro that oxygen concentration is sensed by stem cells and low PO_2 can radically modify their phenotypes. These facts suggest that monitoring of PO_2 in cell culture could be very important to application of cells.

Standard methods of monitoring pO_2 in vitro are using the Clarke-type electrodes (Clark and Lyons 1962, Hahn C E W 1996 and Oqino H 1995). The electrochemical sensors comprise a cathode and anode, surrounded by an electrolyte and a membrane that is permeable to oxygen. When oxygen diffuses through the membrane and electrolyte to reach the cathode, it reduces to hydroxide ions. The cathode donates electrons to the oxygen molecules and the current that flow between cathode and anode is proportional to the oxygen partial pressure in the sample. This electrode itself consumes oxygen so the pO_2 reported will be lower than that seen by cells in the absence of the electrode. And also the sensor shows some sensitivity to nitrous oxide (Ward 1985, Gore 2000). In one study (Pettersen *et al* 2005), microelectrodes were used to monitor the pO_2 in cell cultures under static conditions, significant differences between measured peri-cellular oxygen tension and predicted oxygen tension was found in ambient air. Another reported method is to use oxygen sensitive fluorophores dissolved in the medium for pO_2 measurement (Swartz 2007), but the sensitive fluorophore may be toxic to the cell.

The above sensing methods have some disadvantages such as the high expense, the limited lifetime and slow time response of electrochemical sensors. Especially, the sensors have been unsuitable for continuously monitoring a change of pO_2 in cell culture because for an electrochemical sensor the device itself consumes oxygen as well and for media dissolved with oxygen sensitive fluorophores the tissue culture system needs to be removed from the incubator to read signal. To overcome these disadvantages, optical oxygen sensors (Demas *et al* 1999, Chen *et al* 2012) based on the oxygen fluorescence quenching provides another option.

Here we report a novel fibre optic oxygen sensor for monitoring of pO_2 in cell culture. In this study a platinum Pt(II) complex, Platinum-Octaethyl-Porphyrin (PtOEP), was used as the oxygen sensing luminophore material and a polymer, Poly(ethyl methacrylate) (PEMA), was used as a matrix to immobilize the luminophore. A cylindrical-core fiber based optic oxygen sensor was fabricated for measuring changes in oxygen partial pressure in liquid phase. The fibre sensing element was made by coating a decladded silica core/polymer cladding optical fibre with a sensing matrix layer that contained Pt (II) complex and had a higher refractive index. The excitation light is therefore guided into the cylindrical-core waveguide structure and interacts strongly with the luminophore in the sensing layer, even if the

thickness of the layer is thin. The performance of the fibre optic oxygen sensor was evaluated in gas phase and then calibrated at different pO_2 level in water before used for cell culture pO2 measurement. The results showed that the sensor is capable to measure pO_2 in both gas and liquid phases.

The experiment of cell culture pO_2 monitoring was carried out using porcine kidney (PK-15) cells. The experimental result showed that the higher cell density, the lower pO_2 in liquid phase under a condition of ambient air environment. This could be especially true at condition of high cell density and low pO_2 in gas phase environment, substantiating the need for monitoring and controlling of pO_2 in liquid rather than gas phase.

2. Principle and method

2.1 Principle

The optical oxygen sensor is based on luminescence quenching by oxygen. In dynamic quenching, the luminescence intensity and luminescence life time are related to the oxygen concentration according to the Stern-Volmer relation:

$$I_0 / I = \tau_0 / \tau = 1 + Ksv[O_2] = 1 + k\tau_0[O_2]$$

where I_0 and I are the intensities of the luminescence in the absence and presence of oxygen respectively; τ_0 and τ are the lifetime of the excited state luminescence in the absence and presence of oxygen respectively; Ksv is the Stern-Volmer quenching constant; $[O_2]$ is the oxygen concentration; k is the bimolecular rate that describes the efficiency of the collision between the luminophore and oxygen molecules. Under ideal conditions, the plot of $(I_0/I - 1)$ or $(\tau_0/\tau - 1)$ against $[O_2]$ is linear with a slope equal to Ksv, and can be used for simple sensor calibration.

2. 2 Fiber optic oxygen sensor

Sensor Fabrication: In this work, Platinum-Octaethyl-Porphyrin (PtOEP) and Polyethylmethacrylate (PEMA) were used as the luminophore and the polymer matrix material respectively. Dichloromethane was used as the solvent to dissolve luminophore and polymer matrix. For the preparation of forming the sensing element, the luminophore-doped polymer solution was made by dissolving 1 mg PtOEP and 100 mg PEMA with 1 ml dichloromethane a 1.5 ml amber glass bottle sealed with a polypropylene lid. The mixture was stirred and this ensured PEMA and PtOEP dissolved in solvent completely. Prior to the process of dipping-form sensing tip, the fluoride polymer cladding at the end section of a pigtailed multi-mode silica fibre (OFS Fitel, 200HCS® Low OH High Numerical Aperture 0.43 NA Fibers, 2.5 m in length and 200 μm in core diameter**)** was removed and then cleaned with isopropyl alcohol and cleaved. The decladded

end section of the fiber was dipped into the luminophore-doped polymer solution about 10 mm and then withdrawn from the solution. A sensing film was formed at the end section of the fiber as the solvent quickly evaporated. Then the sensing element was dried in ambient air for at least 24 h. During the dipping process the luminescent signal from the sensing element was monitored using the experimental set up shown in Figure 1.

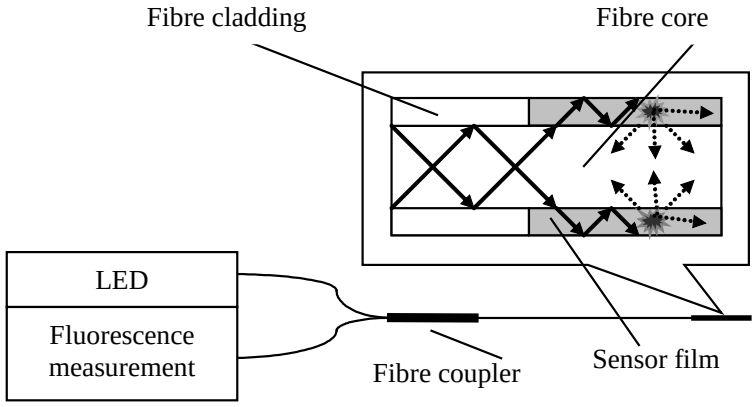

Figure 1 Schematic of experimental set-up

Figure 1 shows the schematic illustration of the experimental set-up for luminescent signal monitoring during the sensor dipping process.

2.3 Cell sample preparation

The porcine kidney cells (PK-15 cells) were maintained in complete medium (minimal essential medium) supplemented with 10% heat-inactivated fetal bovine serum (PAN Biotech) at 37° with 5% CO_2 in an incubator. PK-15 cells grown in complete medium and passaging was routinely performed every 2-3 days (as appropriate) at 70-80% confluence.

The cells in three 25-cm^2 cell culture flasks were divided into five cells samples, which have five different cell concentrations (for 0 mL, 1 mL, 2 mL, 3 mL and 4 mL cells suspension) when they reached 90%-100% confluence. Among there the cells in every 25 cm^2 cell culture flasks were digested with 1 mL 0.25% trypsin for about 5 minutes and then re-suspended in 5 mL Dulbecco's modified Eagle's medium(DMEM).

3. Experiments and results

3.1 Evaluation of sensor performance

Sensor sensitivity: the experimental set-up for evaluating sensor sensitivity was previously reported in (Chen et al 2012). The sensor was evaluated in gas phase. In the experiment, nitrogen and oxygen from gas cylinders were mixed and the

mixing ratio was controlled using a precision gas mixing pump (Wostoff, Germany) before flowing into the gas testing chamber. The oxygen concentration, which was set from 0 to 100% O2 at a room temperature of 20 ºC, was also measured using an oxygen analyzer (Servomex OA570). During testing the fiber optic oxygen sensor was inserted into the testing chamber and the life time of the luminescent light from the sensor head was measured using the phase measurement system (NeoFox, Ocean optics) and recorded in a computer. In the luminescence phase measurement system (NeoFox, Ocean Optics Inc, USA) a modulated UV light from LED integrated in the system was launched into the sensor tip via the one arm of the optical fiber coupler and the luminescent light emitted from the sensor tip was collected through the other arm of optical fiber coupler. The modulated UV excitation light at a preset frequency (0.98 kHz) interacts with the lumeniphores in the sensing element and the resultant fluorescence signal (also at 0.98 kHz) is delayed by a phase angle, ψ. The ratio of tan(ψ), at any given oxygen concentration, to that measured in the absence of oxygen, tan($\psi 0$) , is equal to $\tau/\tau 0$, therefore providing a way of measuring the oxygen concentration independently of the signal intensity, which is affected variably by ambient light intensity changes. The experiment was carried out at room temperature and one atmosphere pressure. Figure 2 shows the continuous change of luminescence lifetime measured while the oxygen concentration in the testing chamber change from 0% to 5%, 10%, 20%, 30%, 40%, 50%, 60%, 80% and 100% respectively.

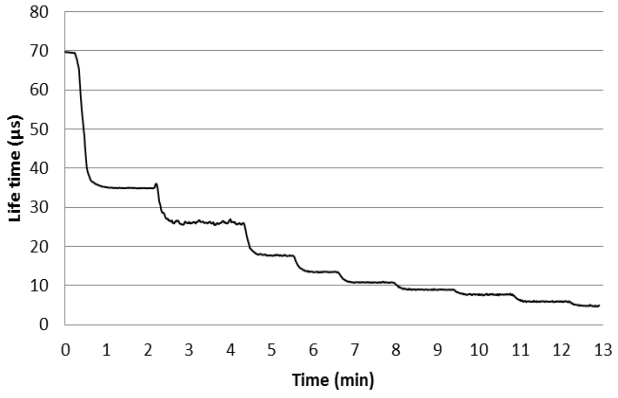

Figure 2 Change in luminescence lifetime versus oxygen concentration (dashed line) and Stern-Volmer plots (solid line) for oxygen sensor in the gas test.

Figure 3 shows the luminescence lifetime measured with the fibre optic oxygen sensor versus the oxygen concentration in the testing chamber. And Figure 3 also shows a Stern – Volmer plot for the (τ/τ_0-1) of the sensor with a linear relationship to the oxygen concentration from 0% to 100%. This plot shows that the sensitivity of the sensor is around 13.5 over the range 0-100% O_2.

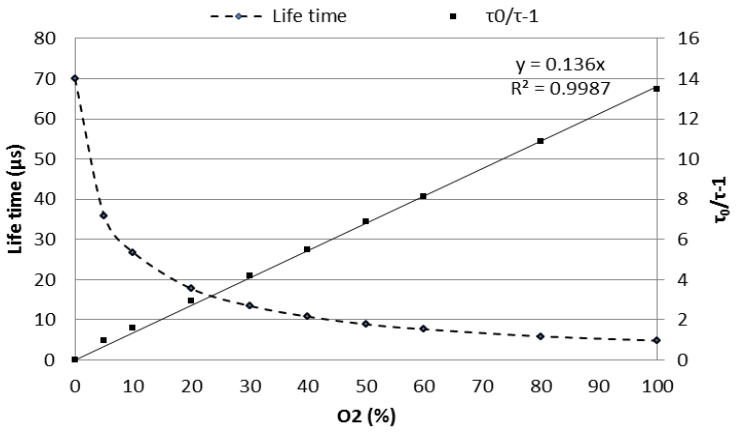

Figure 3 Change in luminescence lifetime versus oxygen concentration (dashed line) and Stern-Volmer plots (solid line) for oxygen sensor in the gas test.

The photo stability of the fibre optic sensor was tested by continuously measuring the life time of the luminescence light from sensing element on the sensor for over 4 hours in room air environment. Figure 4 shows that the life time of the fluorescence measured during the 4 hours test. The result shows the sensor was stable and the variation of the life time of luminescence measured was less than 0.39µs in 4 hours.

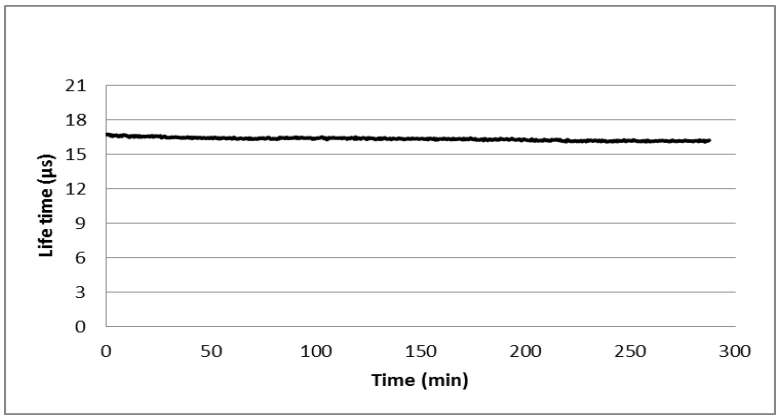

Figure 4 Change in luminescence lifetime in 4 hours photo stability test

3.2 pO2 measurements in cell culture

Sensing system calibration: to meet the application environment the fibre optic sensor was calibrated in a liquid testing bottle, with the luminescence phase detection system. During the test, water was used as the test liquid medium and the oxygen sensor tip was inserted into the liquid in the testing bottle, which was set in a water bath with a temperature at 37 ºC and bubbled with O_2/N_2 mixed gases. The mix ratios of oxygen and nitrogen were 0/100, 2/98, 5/95, 15/85, 21/79 and 40/60 respectively, which results in the pO2 in the testing liquid at 0, 2, 5, 15, 21, 40 kPa respectively. Figure 5 shows the

change of luminescence lifetime measured with the fibre optic oxygen sensor versus the change in pO2 in the testing liquid at 37 ºC. And Figure 5 also shows a Stern – Volmer plot for the (τ/τ_0-1) of the sensor with a linear relationship to the change in pO2. This plot shows that the sensitivity of the sensor is around 8.5 over the range 0-40% O2.

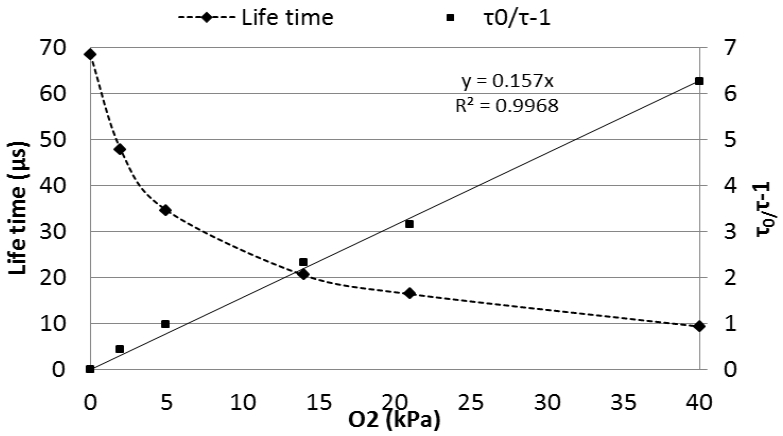

Figure 5 Change in luminescence lifetime versus pO2 (dashed line) and Stern-Volmer plots (solid line) for oxygen sensor in the liquid test at 37 °C.

Cell culture pO2 monitoring: Measuring of pO_2 in cell culture was carried out in cell culture laboratory. During the test the fiber optic sensing tip was positioned in the cell culture flasks and closed to the cells at the bottom of flasks, which were placed in an incubator at the temperature of 37 ºC and contained five different concentrations of PK-15 cell respectively. The pO_2 in each flask was measured using the luminescence phase measurement system (NeoFox) and recorded in a computer. Several dada points were taken for each measurement and the pO_2 level was obtained by averaging of each set of data measured. Figure 6 shows the sets of pO_2 data recorded for five different cell concentrations at different times.

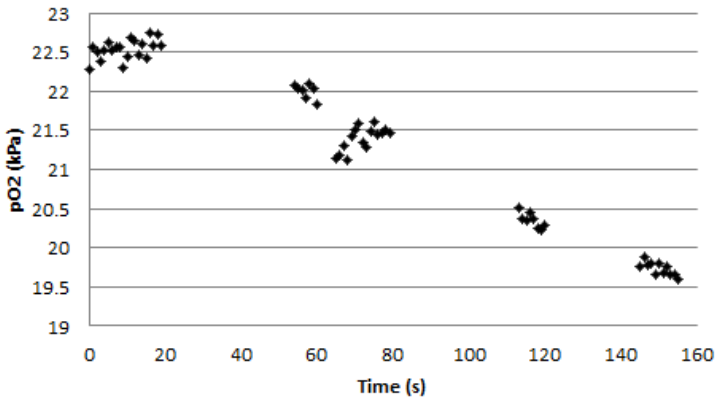

Figure 6 The pO_2 measured using fiber optic oxygen sensor at different PK-15 cell concentrations in cell cultures

3.3 Cell counts

After Measuring of pO_2 in five cell culture samples, the cells samples were digested with 1 mL 0.25% trypsin for about 5 minutes and then re-suspended in 5 mL DMEM. Take approximately 20 μL cells suspension to cell counting chamber (for Counter Star) for each cells sample. Then the amounts of cells can be estimated by Counter Star and the cell concentration was calculated for each cells samples. Table 1 showed the pO_2 level measured using fiber optic oxygen sensor at each PK-15 cell density in the cell culture flasks.

Table 1 pO_2 level at different cell concentrations

Flask Number	Cell concentration (Cells/ml)	pO_2 level (kPa)
1	0	22.28
2	0.35×10^6	22.08
3	0.92×10^6	21.15
4	1.25×10^6	20.51
5	1.46×10^6	19.77

4. Conclusion

The experiments demonstrate the monitoring of peri-cellular pO_2 for PK-15 cells in cell culture using fiber optic oxygen sensor. The results clearly showed that the level of pO_2 in liquid phase in cell culture is not only dependent on the pO2 in gas phase in culture environment but also on cell concentration and cell metabolism. Therefore there is a need of monitoring and controlling the pO_2 level in the cell culture to maintain cultures at physiological conditions.

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