Development of fiber optic fluorescence oxygen sensor in both in vitro and in vivo systems

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Abstract

The accurate measurement of arterial blood oxygen partial pressure often plays an important role in the clinical assessment of patients with respiratory conditions such as an acute exacerbation of chronic obstructive pulmonary disease and acute lung injury/adult respiratory distress syndrome. An oxygen-sensitive fluorescence indicator with high biocompatibility was synthesized and then fabricated to the end of an optical fiber. The properties and accuracy of this oxygen sensor were investigated in vitro using physiologic solutions under varying conditions or human blood, and in vivo by obtaining measurements after inserting this optical sensor into the collateral circulation system of rabbits. The sensitivity of the oxygen sensor was relatively stable during altered conditions of pH, P\textsubscript{CO\textsubscript{2}}, osmolality, and protein concentration in solutions and during alterations of oxygen and nitrogen content in human blood. There was a linear correlation between the reciprocal value of the fluorescence intensity and PaO\textsubscript{2} in both in vivo and in vitro experiments (animal arterial circulation and human blood). Our results suggest that this oxygen-sensitive fluorescence indicator, which has a high biocompatibility, may have the potential for use in real-time monitoring of blood oxygen partial pressure in various clinical settings.

Keywords: Oxygen sensor; Fluorescence; Optical fiber; Quenching; Oxygen partial pressure

1. Introduction

The arterial oxygen partial pressure (PaO\textsubscript{2}) has an important role in the clinical assessment of patients with both acute exacerbation of chronic obstructive pulmonary disease (COPD) and acute lung injury/adult respiratory distress syndrome (ARDS) (Tsai et al., 2004). Although a number of methods are currently used in the clinical setting to measure the levels of PaO\textsubscript{2}, including blood gas analysis, oxygen electrodes or transcutaneous oximetry (Harsten et al., 1988; Mahutte, 1998; Scott et al., 1971), some practical issues with these modalities have been raised by clinicians, e.g. the distance of the analyzer from the bedside, the need for special training of clinicians and staff for proper blood sample collection and handling, the invasive nature of arterial blood sampling, potential contamination of blood samples by the biological media used in processing, the sensitivity of the method used, and others. Thus, there is an unmet need to develop more practical and sensitive methodologies for an accurate assessment of systemic oxygenation.

Fluorescent quenching measurements offer an attractive alternative to more commonly used technologies. In principle, there is a linear relationship between the ratio of fluorescent intensities of a substance in the absence and the presence of a quencher, and with alterations in the concentration of the quencher – that is if the quencher is readily and equally available to the fluorophore. Many current technologies measure the partial pressure of oxygen (P\textsubscript{O\textsubscript{2}}) by relating the loss of fluorescence to the amount of oxygen present. It has been suggested that the use of luminescence intensity for calculating P\textsubscript{O\textsubscript{2}} is a simple problem in signal processing, and suitable for oxygen-sensitive monitoring (Fujiwara and Amao, 2002, 2003a,b; Ishiji and Kaneko, 1995; Lee et al., 1987; Lubbers...
Studies were designed to examine the utility and accuracy of a substrate used as an oxygen fluorescent indicator in both in vitro and in vivo experimental systems. The polymer material, 2-methacryloyoxyethyl phosphorylcholine (MPC) was selected based on its high biocompatibility and previous successful use in the human body (Kihara et al., 2003; Mang et al., 2005). This compound appears to have excellent blood compatibility due to its properties of reduced protein adsorption (Ishihara et al., 1992), inhibition of platelet adhesion (Kojima et al., 1991) and decreased thrombus formation (Ishihara et al., 1990) on its surface.

Due to the lack of readily available optical sensors to measure oxygenation in both animals and humans (Gehrich et al., 1986; Tsukada et al., 2003), the aims of the present study were to validate a ruthenium-based oxygen sensor for measuring oxygen in bioreactors, and to investigate this device’s oxygen sensing properties during conditions of varied temperature, pH, and other specific interference factors. The effectiveness and accuracy of this oxygen sensor were investigated in vitro using physiologic solutions under varying conditions or human blood, and in vivo by obtaining measurements after inserting this optical sensor into a collateral circulation system from the carotid artery of rabbits.

2. Materials and methods

The study protocol was approved by the institutional review board for research on human materials and the local ethical board for animal research of the Zhongshan Hospital of Fudan University.

2.1. Oxygen sensor probe

The optical oxygen sensor consisted of optical fiber, sensor probe film attached to the distal end of the optical fiber and a catheter tube with schematic illustration as shown in Fig. 1A. The sensor probe film was prepared by casting of a tetrahydrofuran (THF) solution of fluorescence dye and an acrylate copolymer, which was synthesized with free radical poly-

Fig. 1. A Schematic illustration and micrograph of an optical oxygen sensor. (A) Schematic illustration of the oxygen sensor. An optical fiber 3.5 mm in diameter was inserted into a catheter tube 4.0 mm external diameter. A MCP copolymer-doped fluorescence dye was fixed at the edge of the catheter. (B) Micrograph of the oxygen sensor. Excitation and emission spectrum of fluorescence dye doped MPC copolymer of the oxygen sensor. (C) The block diagram of the measuring equipment. Arrowheads of solid lines and broken lines show the light path of light or fluorescence, and the electrical signals, respectively.
merization of n-butyl methacrylate (BMA) and MPC (Patent pending no.: China 200410053454.1, USA US11/196,305, Japan 2005–226678). This biomimetic polymer matrix had a high permeability to water and oxygen and anti-protein adhesion properties. An optical fiber at diameter of 3.5 mm was inserted into a catheter tube with an external diameter of 4.0 mm. A MPC copolymer-doped fluorescence dye was fixed at the edge of the catheter. The excitation and emission spectrum of this fluorescence dye doped MPC copolymer oxygen sensor probe film had a maximal absorption at 490 nm and an emission peak at 640 nm, as shown in Fig. 1B.

2.2. Instrumentation and data processing

A blue light emitting diode (LED) served as the illumination source for the oxygen detection system (Fig. 1D). The fluorescence from the oxygen sensor probe was monitored continuously by a fluorescence microscope using a magnification of ×10 and a filter set consisting of a 480 ± 10 nm excitation filter, a 510 nm dichroic mirror, and a 660 ± 10 nm emission filter. Signals were detected by a photomultiplier tube (PMT) (H120, Hamamatsu, Japan), amplified, digitized and then recorded by an analog-to-digital converter (A/D, Duo.18, World Precision Instruments, USA) at a rate of 5 Hz.

2.3. Application in vitro

A solution circulating loop system was settled to block schematic diagram, as shown in Fig. 2A. Varying concentrations of oxygen were infused into the solutions studied. Two pieces of PE-90 tubing were connected to the loop of the container where a pump drove the circulation of the solution. The oxygen sensor was inserted into the tubing of the loop in order to detect the oxygen tension in the container. Oxygen homeostasis is continuously changing in patients with critical illness due to multiple and frequent pathologic and physiologic alterations, e.g. the plasma osmolarity disturbances, acid–base imbalance, hypercapnia and fever et al. Therefore, we investigated the fluorescence properties and the accuracy of the experimental oxygen sensor in conditions of hyperosmolality, isosmolality, hypoosmolality. In order to establish hyperosmolality, the solution used in the circulating loop system was Ringer’s solution containing 300 mM sucrose – this yielded an osmolality of 500 mosmol/kg H2O. For isosmolality, Ringer’s solution was used without sucrose, yielding an osmolality of 300 mosmol/kg H2O. For hypoosmolality, Ringer’s solution was diluted with water to achieve an osmolality of 200 mosmol/kg H2O. The pH of these solutions was 7.4. The potential influence of colloidal pressure was evaluated by using Ringer’s solutions containing different percentages of bovine serum albumin (BSA), e.g. 2.5, 5.0 and 7.5%, respectively. The accuracy of the experimental oxygen probe was also tested by varying other conditions: the pH from 6.0, 7.0 to 8.0, the viscosity of solutions containing 5 and 10% sucrose, the temperature from 35 to 41°C, and altering the Pco2 with or without 100% oxygen.

2.4. Application in vivo

Adult male and female rabbits, weighing 2.7–3.3 kg, were purchased from Animal Center of Fudan University (Shanghai, China) and used in these experiments. Animals were anesthetized with intravenous injection of pentobarbital sodium (30 mg/kg) followed by a constant infusion at 4 mg/kg/h throughout the experiment. After the trachea was intubated with a cuffed endotracheal tube (i.d. 1.0 mm, o.d. 1.5 mm), the rabbits were stabilized for 30 min before commencing the experiment. The animals were ventilated with an AVEA comprehensive ventilator (VIASYS, USA), at a frequency of 20 breaths/min, tidal volume of 10 ml/kg, and inspiratory time fraction of 1:2, PEEP 2 cm H2O. The right external carotid artery was cannulated using the polyethylene PE-90 tubing with two ends, in order to establish a collateral circulation system (Fig. 2A). A PE-200 tubing, in which the oxygen sensor probe was inserted, connected two pieces of PE-90 tubing, allowing arterial blood to flow passed the probe via this collateral circulation system in the same manners if the probe was directly inserted into the carotid artery. As a control, arterial blood was simultaneously sampled from the left external carotid artery for blood gas analysis.

2.5. Application in human blood samples

The experimental oxygen sensor and the circulating loop system described above were used to determine the partial pressure of oxygen in arterial blood obtained from healthy volunteers – arterial blood was obtained using a standard arterial blood gas kit (Fig. 3A). The influence of oxygen and nitrogen on the fluorescence of the sensor was investigated by infusing different concentrations of these gases into the blood samples – oxygen or nitrogen was infused separately and not simultaneously.

2.6. Statistical analysis

The results are presented as mean ± S.D. Intragroup comparisons were assessed by one-way analysis of variance and by the t test for paired samples. The unpaired t test was used to test differences between groups. Coefficient of correlation was analyzed. p < 0.05 was considered significant.

3. Results

The influence of varying nitrogen or oxygen concentrations in the circulating loop system is shown in Fig. 2B. The fluorescence readings from the sensor did not change with alterations in the concentrations of saline (0.45, 0.9 and 1.8%), sucrose, or albumin (2.5, 5 and 7.5%), as shown in Fig. 2C. The time it took for oxygen to separate from the dye when the sensor was exposed to the nitrogen was named “separation time”, which related to the increasing curve. The time it took for oxygen to combine with the dye when the sensor exposed to the oxygen was called “combination time”, which related to the decreased curve. Changes in osmolality, colloidal pressure, pH, partial pressure of CO2, and viscosity had no influence on the “combination” and “separation” times (p > 0.05).
Fig. 2. Application in a circulating loop system. (A) Schematic showing the circulating loop system. Different concentration oxygen was infused into the solutions, and two PE-90 tubing connected the container and the PE-90 tubing into a loop. A roller pump drove the solution circulating in the loop. The oxygen sensor was inverted into the tubing of the loop and detected the oxygen tension in the container. The solid arrowhead showed the direction of solution circulation. (B) The fluorescence properties of the sensor in different states: (1) hyperosmolality, isosmolality, hypoosmolality; (2) different colloidal pressure; (3) pH from 6.5 to 8; (4) with and without CO2; (5) viscosity. The representative curves were shown. There was no effect on the curve when exposed to these different states. (C) The time from oxygen separated from the dye when the sensor was exposed to the nitrogen was named “separation time”, which related to the increasing curve; and the time oxygen combinates with the dye when the sensor exposed to the oxygen was called “combination time”, which related to the decreased curve. The osmolality, colloidal pressure, pH, CO2, and viscosity have no influence on the combination and separation time ($n=6$, $p>0.05$). (D) The effect of the temperature was shown. With the increase of one centri-degree, the fluorescence decreased 0.8%. The solid arrowhead showed the direction of solution circulation. ($n=6$, $r=0.9258$, $p<0.01$). (E) The fluorescence intensity was very stable during 8 h record.

To investigate the potential influence of pH on this system, measurements were made in solution where the pH varied from 6.0 to 8.0. This pH range was selected in order to simulate pH changes that occur clinically in critically ill patients. Variance of pH had no effect on the fluorescence measurements of the oxygen sensor, as shown in Fig. 2B and C. Additional measurements were made in a N2 solution, before and after CO2 was infused into the experimental solutions, to mimic the physiologic consequences that occur in patients with hypo-ventilation, hyper-ventilation, and disorders of acid-base metabolism (e.g. respiratory acidosis and respiratory alkalosis). Variance of PdCO2 had no effect on the fluorescence measurements of the oxygen sensor, as shown in Fig. 2B and C.

Exposure to nitrogen increased the data curve, while exposure to oxygen decreased the data curve. Variation in the temperature of the solutions – from 35 to 38 and 41°C – decreased the fluo-
Fig. 3. Application in rabbits. (A) Schematic showing a collateral circulation in which the artery inserted with the tubings and oxygen sensor. The right external carotid artery were cut off and cannulated with two polyethylene PE-90 tubing in its two ends respectively. A PE-200 tubing, in which the oxygen sensor probe was inserted, connected the two PE-90 tubings and the artery blood passed the probe as the same effect as the probe inserted in the artery. (B) The represent curve showed: when the rabbit was ventilated with different concentration oxygen, the fluctuant fluorescence intensity changed as the FiO2 fluctuated. (C) In hypoxia, the light change was showed. As the FiO2 decreased from 21 to 2.5% step by step, the fluorescence increased 60% gradually. (D) The correlation between the value \(I_0/I-1\) and the PaO2 indicated there was a line relation between them. The \(R\) value was 0.9356 (\(n=29, p<0.01\)).

Fluorescence about 2.5 and 5%. The fluorescent intensity decreased about 0.8% for each degree change (Fig. 2D), showing a linear relation between the temperature and the measured fluorescence intensity (Fig. 2E).

In rabbits, the fluorescence intensity decreased in response to ventilation with oxygen at different concentrations – FiO2 was increased incrementally from 0.21 to 1.0 (Fig. 3A). Time course data is shown in Fig. 3B. Fluorescence intensity fluctuated as the FiO2 was incrementally increased from room air (0.21) to 100% oxygen (FiO2 of 1.0). A 10% increase in FiO2 correlated with a 6% decrease in the fluorescence intensity. Interestingly, if the animal was ventilated with room air after being on a FiO2 of 1.0, the fluorescence returned to the same level observed at the start of the experiment, when room air was being ventilated. While adjusting the FiO2, the fluorescence intensity began to change within 15 s, and readings stabilized by 2 min. When the FiO2 was decreased (2.5–21%), the fluorescence intensity changed in an inverse proportion to the FiO2, as shown in Fig. 3C. Under conditions of hypoxia, the correlation between the fluorescence intensity \(I_0/I-1\) and the PaO2 appeared to be linear with an \(R\) value of 0.9356 (Fig. 3D) – with relative arterial blood oxygen partial pressure (PaO2) being concurrently measured by blood gas analyzer.

In order to check the stability of the sensor in vivo, photobleaching was utilized and the fluorescence intensity was continuously recorded – that is the oxygen sensor was exposed to blue light continuously for 8 h, after which the fluorescence intensity measurements decreased 1–2%. Additionally, bacterial and viral cultures of the sensor were negative during 5–7 catheterization days.

Fig. 4. Application in human blood samples. (A) With oxygen and nitrogen infused into the blood samples alternately, there were the same curves as performed with the nature saline solutions. (B) The fluorescence intensity of some blood samples with different PaO2 from health volunteers (red point) (\(n=27, r=0.9168, p<0.01\)). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)
The fluorescence intensity of blood obtained from healthy volunteers was measured in vitro at different levels of \( \text{PaO}_2 \). The curves generated during the infusion of human arterial blood with oxygen or nitrogen was similar to those previously observed in the above experiments which utilized saline (Fig. 4A). The relationships between the fluorescence intensity and the oxygen partial pressure in these experiments were similar to those described above in the in vivo rabbit experiments (Fig. 4B).

4. Discussion

The accurate measurement of arterial blood oxygen partial pressure is often critical in the clinical assessment of patients with respiratory conditions such as an acute exacerbation of chronic obstructive pulmonary disease and acute lung injury/adult respiratory distress syndrome, and directly correlates to the pulmonary function of these patients with critical illness. The development of an efficient and accurate implantable oxygen sensor is still a great challenge (Frost and Meyerhoff, 2002; Mahutte, 1998). A variety of prototype catheter-style commercial devices have been developed to date for experimental intravascular blood gas sensing purposes (Fogt, 1990; Meyerhoff, 1993). However, most of these devices are not practical nor useful in the clinical arena, primarily because they are inaccurate generating erratic results of devices are not practical nor useful in the clinical arena, primarily because they are inaccurate generating erratic results of interference characteristics. This catheter-based fluorescence system was developed to detect and record the fluorescence intensities measured on a computer. The changing conditions of blood osmolality, viscosity, colloid osmolarity and acid–base balance which occur frequently in patients with critical illnesses were mimicked in vitro in the present study in order to detect the sensitivity and accuracy of the oxygen sensor during abnormal as well as normal physiologic conditions. Our results demonstrated that the sensor could sensitively detect the changes of the oxygen partial pressure in varying conditions of osmolality, colloidal pressure, viscosity, \( \text{pH} \), \( \text{pCO}_2 \), and temperature, all critically important factors which have been shown to markedly influence the measurement of oxygen partial pressure in previous studies. The long response times observed might be related to time delay and capacitance of the recording chamber. In vivo, the sensor could sensitively detect a variety of arterial oxygen tensions from 35 to 500 mmHg. The \( I_0/I − 1 \) value was directly proportional to oxygen partial pressure measured by blood gas analyzer, used as a standard, with a clear correlation. The accuracy and reproducibility of these data indicate the potential use of this sensor for routine clinical application. This oxygen sensor yielded consistent results over an 8 h continuous detection period, and photobleaching of the sensor did not significantly alter the data generated. Furthermore, the sensor could detect a wide range of oxygen partial pressures (from about 760 mmHg to 0) in human blood, which would be of use clinically. It is also possible to calculate the \( \text{PaO}_2 \) according to the fluorescence intensity data generated by this device.

Of note, the response time of this sensor is less than 15 s, suggesting that is could be very useful in the clinical setting via the generation of oxygen-sensitive recording of dynamic changes in the \( \text{PaO}_2 \). The results of these studies also indicated that the oxygen sensor can detect the \( \text{PaO}_2 \) during conditions of both in hyperoxia and hypoxia – thus it could be utilized as a bioinstrumentation for oxygen pressure monitoring. Previous studies have focused on oxygen sensors utilizing different materials for oxygen sensing, and most of these studies have only been in vitro – only a few studies have been performed with devices in in vivo systems (Gehrlich et al., 1986; Sinaasappel and Ince, 1996; Tsukada et al., 2003). The results from animal experiments performed in the present study demonstrated that the optical oxygen sensor studied, which using fluorescence intensity as an indicator, can accurately monitor the \( \text{PaO}_2 \), in vivo. Furthermore, the \( I_0/I − 1 \) values generated by this device from arterial blood obtained from rabbits during conditions of hyperoxia and hypoxia closely correlate with \( \text{PaO}_2 \) values from arterial blood obtained simultaneously and analyzed by a standardized blood gas machine.

In conclusion, we developed a new optical oxygen sensor device to measure alternations of oxygenation in both in vitro and in vivo systems. The performance of this sensor which is made with a biocompatible MPC-based membrane, generated accurate and consistent measurements of arterial blood oxygen partial pressure during alterations of multiple physiologic conditions. The sensor was shown to be extremely sensitive in detecting changes of oxygen partial pressure and had promisingly short response time in detecting these changes. Such an oxygen indicator with a high bio compatibility will potentially be useful for continuously or intermittently monitoring the partial pressure of oxygen not only in animal in vivo experimentation, but perhaps after further clinical trials, in the clinical setting for monitoring patients.

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