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Real-Time, In Vivo Determination of Dynamic Changes in Lung and Heart Tissue Oxygenation Using EPR Oximetry

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Abstract

The use of electron paramagnetic resonance (EPR) oximetry for oxygen measurements in deep tissues (>1 cm) is challenging due to the limited penetration depth of the microwave energy. To overcome this limitation, implantable resonators, having a small (0.5 mm diameter) sensory loop containing the oxygen-sensing paramagnetic material connected by a pair of twisted copper wire to a coupling loop (8–10 mm diameter), have been developed, which enable repeated

measurements of deep-tissue oxygen levels (pO_2 , partial pressure of oxygen) in the brain and tumors of rodents. In this study, we have demonstrated the feasibility of measuring dynamic changes in pO_2 in the heart and lung of rats using deep-tissue implantable oxygen sensors. The sensory loop of the resonator contained lithium octa-n-butoxynaphthalocyanine (LiNc-BuO) crystals embedded in polydimethylsiloxane (PDMS) polymer and was implanted in the myocardial tissue or lung pleura. The external coupling loop was secured subcutaneously above chest. The rats were exposed to different breathing gas mixtures while undergoing EPR measurements. The results demonstrated that implantable oxygen sensors provide reliable measurements of pO_2 in deep tissues such as heart and lung under adverse conditions of cardiac and respiratory motions.

Keywords

Oximetry; Electron paramagnetic resonance (EPR); Implantable oxygen sensors; Myocardial tissue; Lung pleura

1 Introduction

Electron paramagnetic resonance (EPR) oximetry enables measurement of partial pressure of oxygen (pO_2) in biological tissues [1]. However, use of EPR oximetry for in vivo measurements, particularly in deep-seated tissues is limited by the depth of penetration of microwave energy. This limitation was addressed by the Swartz group through the development of implantable resonators consisting of an oxygen-sensory loop linked to a coupling loop [2–4]. The implantable oxygen sensors have been demonstrated for temporal oximetry measurements in tumors and brain in various animal models, as well as single time-point measurements in the heart [2–4]. As yet, temporal studies of heart and lung tissue pO_2 using the implantable oxygen sensors have not been reported. Measurements of dynamic changes in cardiac or lung tissue pO_2 on exposure to hyperoxia including hyperbaric therapy or periodic administration of oxygen (oxygen cycling) also present a significant challenge. The goal of this study was to test the implantable oxygen sensors for reliable and repeated measurements of oxygen concentration in the heart and lung, in a rodent model.

2 Methods

2.1 Fabrication of Implantable Resonators

The implantable resonators were fabricated in-house using 34-gauge copper wires (Fig. 11.1). One end of the copper wire was used to create a sensory loop (0.2–0.5 mm diameter) while the other end was rounded to create coupling loop (9 mm diameter). The distance between the sensory loop and coupling loop was 7–10 cm. The sensory element of the tip was created by mixing LiNc-BuO (lithium octa-nbutoxynaphthalocyanine) oxygen-sensing crystals [5] in uncured polydimethylsiloxane (PDMS; 1 part cure accelerant: 10 parts base, w/w; Factor II, Inc.) and dipping the sensory loop of the implantable resonator in the mixture. The resonator/sensor was then placed in an oven at 80 °C to cure overnight. The following day, the unit was removed, and coated by immersing in a PDMS:heptane (1:3 w/w) dispersion with an additional overnight cure at 80 °C.

2.2 Implantation of Resonators for Heart and Lung Oximetry

Sprague-Dawley rats were anesthetized and a thoracotomy was performed using the procedures reported previously [6]. As the sensory loop/tip of the implantable oxygen sensors is not sharp, approximately 1-mm long pilot channels were created in the myocardium or lung by gently inserting the bevel of a sterile, 23-gauge needle at 45° angle to the surface of the organ. The sensory loop was then placed inside this channel at the level of mid-myocardium of left ventricle or the parenchyma of left lung lobe. Care was taken to avoid perforation of the wall or organ and associated vasculature. A suture was placed around the juncture between the sensory loop and the surface of the tissue to prevent the sensory loop from becoming dislodged due to cardiac or respiratory motion. The thoracotomy was closed and the coupling loop was secured in the subcutaneous pouch with sutures prior to final closure (Fig. 11.1). For comparative purposes, in a separate group of rats, un-encapsulated LiNc-BuO crystals were implanted using a 25-gauge needle adapted as a positive-displacement delivery device.

2.3 EPR Oximetry Measurements

Rats were anesthetized using 2–2.5 % isoflurane mixed with breathing gas using an induction chamber. Rats with LiNc-BuO probe in the tissues were tested on day 3 post-implant while those with implantable oxygen sensors were tested on day 4 post-implant. Once anesthetized, the rats were moved to a custom-fabricated chamber to breathe normally while being exposed to different oxygen concentrations mixed with isoflurane gas (1.25 %) to maintain anesthesia. Gas mixtures included 100 % oxygen, 95 % oxygen+ 5 % CO₂ (carbogen), 70 % oxygen, 40 % oxygen, 21 % O₂ (room air), and 10 % oxygen balanced with nitrogen. The experimental scheme is shown in Fig. 11.2.

The chamber was designed to permit inductive coupling with the external loop resonator of the EPR unit. The rats were carefully positioned beneath the resonator of a Magnettech L-band (1.2 GHz) EPR unit such that the surface loop of the external loop resonator was located approximately above the coupling loop of the implanted resonator. Spectra were collected while the rats were breathing different gas mixture. The breathing gas was quickly changed using a simple valve system. The line-width of the EPR signal was analyzed by using a curve-fitting program (OxyScope) and a standard calibration curve for the probe was used to obtain the pO₂ values. The procedure was repeated for myocardial tissue oximetry measurements on day 7 post-implantation. One rat having an implantable oxygen sensor for myocardial measurements was imaged on day 10 post-implant by fluoroscopy (GE OEC 9800) to confirm that the implanted sensor was intact and ascertain the location of the sensory tip and coupling loop (Fig. 11.1).

3 Results and Discussion

The pO₂ data obtained from the heart and lung are shown in Fig. 11.3. Each point represents a single pO₂ value that was calculated using OxyScope curve-fitting program. Two observations are worth noting: (a) the measured values of tissue pO₂ changed, as expected, when the inhaled gas mixture was varied; (b) it was not possible to acquire EPR signal from the bare probes implanted in the rat heart or lung under these conditions in the animals.

Because the sensory tip of the implantable sensor moves with the tissues of interest (lung and heart), there is minimal motional artifact by respiration or heartbeat. Figure 11.4 shows the peak pO_2 values collected from the heart and lung on day 4 post-implant under the conditions of changes in breathing-gas. The results indicate an increase in tissue pO_2 in the heart and lung on exposure to hyperoxic gases. It should be noted that, although the myocardial pO_2 in room air-breathing animal was normal, its response to hyperoxygenation was substantially greater compared to that of lung. This could be attributed to the effect of rigidity of the resonator which potentially could affect heartbeat and pO_2 measurement including some local perturbation at the sensory tip of the implant.

4 Conclusion

We have demonstrated the feasibility of dynamic oximetry in the lung and myocardial tissues of animals using implantable resonator technology with L-band EPR spectroscopy. The EPR acquisition had minimal physiologic artifacts due to the heart-beat and respiration. By increasing the length of the implantable resonator, and with the advent of clinical EPR spectrometers, it should be possible to use these devices in large animal models and humans for deep-tissue measurements of oxygen. The implantable oxygen sensors technology could also be applied to pulse oximetry.

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Fig. 11.1.

Measurement of myocardial pO_2 in rat using implantable resonator (oxygen sensor). (a) Photo of an implantable resonator made of twisted copper wire (34 AWG) showing the coupling loop and sensory loop/tip holding the oxygen-sensing LiNc-BuO crystals, embedded in PDMS. (b) An expanded view of the resonator showing PDMS coating of the wire. (c) Fluoroscopy image of implantable resonator in the heart of a rat taken 10 days post-implantation. The coupling loop is buried under the skin (chest). (d) A typical EPR spectrum obtained using implantable resonator. Spectral fitting and residuals are shown at 8 $\times$ amplification

Fig. 11.2.

Experimental scheme for *in vivo* EPR measurements in rats with bare LiNc-BuO particulates or implantable resonator. The rat is exposed to gases with different concentrations of oxygen in the chamber during EPR oximetry

Fig. 11.3.

Tissue pO₂ values in the rats exposed to gases with different oxygen concentrations. Data were obtained using (a) implantable resonator in the heart (day 4 post-implantation); (b) implantable resonator in the lung (day 4 post-implantation). The breathing gases used were: carbogen (95 % O₂/5 % CO₂), room air (21 % O₂), hypoxia (10 % O₂), 40 % O₂, 70 % O₂, and 100 % O₂.

Fig. 11.4.

Peak values of myocardial (a) and lung tissue (b) PO₂, values obtained using an implantable resonator during different inhaled oxygen mixtures, room air (21 % O₂), 70 % O₂, 10 % O₂, and carbogen (95 % O₂). Data are expressed as mean ± SD (N=4)