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Monitoring cardiopulmonary function and progression toward shock: *oxygen micro-sensor for peripheral tissue*

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Abstract

We are developing a robust, minimally invasive device for detecting progression toward hemorrhagic shock in trauma patients. To accomplish this, oxygen micro-sensors are being developed that contain a solution of oxygen sensitive phosphorescent probes within gas permeable tubing attached to optical fibers. These micro-sensors can be inserted into peripheral tissue to accurately measure tissue oxygenation. As the blood volume decreases (hemorrhage), physiological mechanisms progressively restrict blood flow to “non essential” peripheral tissues, redirecting that flow to the essential internal organs. It is hypothesized that the sensors will detect the shut down of peripheral blood flow well before the decreasing blood volume reaches the threshold where multi-organ failure begins. Proactive treatment with volume expanders or blood, guided by peripheral oxygen measurements, would significantly reduce multi-organ failure and other complications in trauma cases.

1 Introduction

According to the Center for Disease Control, trauma is the leading cause of death between the ages of 1 and 44, of both civilians and military personnel. Hemorrhage is responsible for over 35% of pre-hospital deaths and over 40% of deaths within the first 24 hours of arrival to a trauma facility [1]. In the military environment, this can reach up to 90% [2]. Onset of shock occurs when loss of blood leads to insufficient delivery of oxygen to critical central organs. This depresses essential energy metabolism and increases carbon dioxide and other acids in the cells of these tissues and the organs begin to fail.

Recognizing and rapidly treating inadequate oxygen delivery to tissues is a cornerstone of critical care management, but standard methods for objectively quantifying and monitoring deficient oxygen delivery are recognized to be inadequate (see [3]). First responders must identify which patients have insufficient blood volume, and therefore require perfusion, and which patients do not, avoiding fluid overloading of the latter. Early-stage shock due to insufficient blood volume is often difficult to recognize due to compensatory mechanisms in the body that shut down flow to low priority tissues, such as peripheral muscle, in order to maintain flow to the essential central organs such as the brain, heart and lungs [4]. Infusing fluids into all patients regardless of whether they are deficient in blood volume is not a viable solution because volume overload can cause life-threatening pathology. Dilution of the blood due to infusion of resuscitation products can also cause dysfunction in coagulation and this is associated with a 3.5- to 5-fold increase in mortality [5].

We describe a new, minimally invasive, sensor for accurate continuous monitoring of tissue oxygenation that is suitable for clinical use and should provide objective and quantitative measure of the oxygen delivery to tissue.

2 Methods

Measuring oxygen by phosphorescence quenching

Phosphorescence arises when a phosphor is excited to the triplet state by absorption of a photon of light and then returns to the ground state with emission of light (phosphorescence). The excited triplet state may also return to the ground state by colliding with, and transferring energy to, another molecule (quencher) in the environment. The rate of decay of the excited triplet state, phosphorescence lifetime, depends on the concentration of the quenching molecules in the solution. These properties have been used to develop a highly sensitive and accurate method for oxygen measurement by determining the extent of quenching of a phosphorescent probe [6-9]. The phosphorescence lifetime or intensity may be converted to oxygen pressure using the Stern-Volmer relationship:

$$I^0/I = T^0/T = 1 + k_Q T^0 [pO_2] \quad (1)$$

where I^0 and T^0 are the Intensity and lifetime in the absence of oxygen, respectively, I and T are the intensity and lifetime, respectively, at a given value of oxygen pressure (pO_2), and k_Q is a constant describing the frequency of quenching collisions between the phosphor molecules in the triplet state and molecular oxygen. k_Q is a function of the diffusion constants for phosphor and oxygen, temperature and phosphor environment (see [8] for calibration). A frequency domain phosphorescence lifetime measurement instrument [9] with a 635 nm laser diode as the light source and an avalanche photodiode detector was used in the present studies to determine phosphorescence lifetimes and thereby oxygen concentrations.

Phosphorescent oxygen sensors (Oxyphors)

A number of phosphorescent oxygen probes have been described [10-15]. Oxyphor G3, the one chosen for this application, is based on Pd-tetrabenzoporphyrin [16] (Figure 1). Oxyphor G3 is a Pd tetrabenzoporphyrin (PdTBP) modified with generation-3 polyarylglycine dendrons and coated with a layer of peripheral polyethylene glycol (PEG) residues. The dendrimer in G3 folds tightly around the PdTBP core in aqueous media and controls its exposure to oxygen. The phosphorescence quantum yield of G3 is about 2% and the lifetime T^0 is about 270 μ s. The Oxyphor G3 has absorption bands with maxima at 445 nm and 635 nm and the phosphorescence emission maximum is near 810 nm.

Oxygen micro-sensors

In order to facilitate use of the sensors in the clinics, it is important that the Oxyphor is not in direct contact with the body. To accomplish this we have chosen to sequester the solution of Oxyphor in a sealed Teflon AF chamber. This chamber has been made very small and placed on the end of an optical fiber (Figure 2). The optical fiber conducts the excitation light from a 635 nm laser diode to the sample and returns the emitted phosphorescence to the detector. The current prototype chamber uses Teflon tubing with an outside diameter of 500 microns and an inside diameter of 250 microns, placed on the end of a 250 micron plastic optical fiber.

All animal procedures strictly followed the NIH Guide for the Care and Use of Laboratory Animals and have been approved by the Institutional Animal Care and Use Committee of the University of Pennsylvania

3 Results

The oxygen micro-sensors have been extensively tested *in vitro*. The response time measured when sensors are moved between two solutions with different oxygen pressures (such as air saturated and about 15 torr) is slightly less than 1 minute. This is sufficiently rapid to measure most physiological changes in oxygen pressure in tissues. The *in vitro* stability has been measured by placing micro-sensors in air saturated media and measuring the internal oxygen pressure each 15 seconds over periods of up to 16 hours. The measurement to measurement variability was typically less than $\pm 2\%$. The signal to noise was initially near 40 and declined over the 16 hr to about 20. Individual sensors have been constructed and stored for many days without change in sensitivity as long as they are kept from drying out.

The micro-sensors are intended for measuring oxygen in tissue *in vivo*. Preliminary measurements have been made by “piggy backing” them to experiments studying the effects of hypoxic hypoxia and ischemic hypoxia on brain in a newborn piglet model. The sensors were inserted into the tissue while within a thin-walled 21 gage needle and then the needle withdrawn. Figure 3 shows the measurements obtained when the sensor was placed in the striatum of the brain while Figure 4 shows oxygen measurements placed in peripheral muscle (leg). In both cases, measurements were made for 4-6 hours without significant increase in the noise level of the measurements.

4 Discussion

The first generation of oxygen micro-sensors for measurements in peripheral tissue have very good oxygen measurement capabilities. Their function appears well suited for monitoring the status of trauma patients, particularly by first response teams. Because the Oxyphor is in solution, the calibration is absolute, eliminating any need for on site calibration and making the sensors fully interchangeable. Insertion requires minimal technical expertise and can be accomplished in less than 1 minute. Measurements can begin as soon as the sensor is inserted into the tissue. The sensors are stable under measurement conditions and functioned reliably for periods of at least 4-6 hours after insertion into the tissue. The initial oxygen sensors have outside diameters of 500 microns and response times of about one minute, but it is expected that the size will be reduced to about 400 micron outside diameter, allowing insertion through 22 gage needles and providing response times of 30 seconds or less. The phosphorescence lifetime reader used with the micro-oxygen sensors can be made small, less than 12cm x 10cm x 4 cm, light weight, and battery powered, and therefore convenient and flexible enough for use in the field. The data can also be transmitted to a central computer system by building in an appropriate wireless transmitter, although this function has not been implemented in the prototype instruments.

Because there is a great need for better monitoring of trauma patients and generally for patients requiring critical care, extensive efforts are underway to try to fill this need. Much of the effort focuses on using near infrared light to measure hemoglobin saturation (NIR). This is a very attractive approach because the measurements are non-invasive and readily translated into the clinics. The weakness is that the measurements are of small differences in absorption in highly scattering media containing other chromophors. There are several NIR instruments in use or being tested in the clinics, but their predictive value in clinical applications remains to be established. In the case of trauma patients, clinical trials suggest the current generation of instruments provide data about as predictive of multiorgan failure as the base deficit [3,17,18].

The oxygen micro-sensors described in the present paper measure with high accuracy, even at the low oxygen concentrations expected to exist in tissue under conditions of compromised blood flow. They are, however, more invasive than NIR. Whether the higher accuracy will provide sufficient predictive advantage to justify the greater invasiveness remains to be established.

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Figure 1.

Calibration of Oxyphor G3. The measurements are of the phosphorescence lifetime of Oxyphor G3 as a function of the oxygen pressure when it is dissolved in phosphate buffer and in blood plasma at 23°C. The straight line is the best fit to the Stern-Volmer equation. The inset shows measurements of the Oxyphor G3 lifetime in air saturated phosphate buffer measured over a > 72 hour period.

Figure 2.
A schematic drawing of the prototype oxygen sensor on the end of a 250 micron outside diameter plastic optical fiber.

Figure 3.

Oxygen measurements in the striatum of the brain (fig 3) and peripheral muscle (fig 4) of anesthetized piglets. The sensors were inserted into the tissue inside 20 gauge needles and then the needles withdrawn. Measurements were begun within 1 minute of insertion and continued at 15 sec intervals. During the period labeled hypoxia, oxygen in the inspired air was stepwise lowered over a period of 20 min until the oxygen pressure in the brain decreased to 6-8 torr. The brain oxygen level was maintained at that level for 1 hr and then the oxygen in the inspired gas was returned to 21%.

Figure 4.

Oxygen measurements in the striatum of the brain (fig 3) and peripheral muscle (fig 4) of anesthetized piglets. The sensors were inserted into the tissue inside 20 gauge needles and then the needles withdrawn. Measurements were begun within 1 minute of insertion and continued at 15 sec intervals. Two sensors were inserted, one in the back leg (sensor A) and the other in the fore leg (sensor B). Ischemic hypoxia was induced in the brain by ligating both carotid arteries then lowering the oxygen in the inspired gas over a 20 min period to achieve a brain tissue oxygen pressure of 6-8 torr. This was maintained for 1 hr, the ligations removed and O_2 in the inspired gas raised to 21%. At about 15 min intervals, sensor (A) was disconnected and sensor (B) connected. After 4-6 measurements, B was disconnected and A was reconnected.