Published in final edited form as:

Magn Reson Med. 2019 February ; 81(2): 781–794. doi:10.1002/mrm.27445.

Transcutaneous oxygen measurement in humans using a paramagnetic skin adhesive film

Maciej M. Kmiec^a, Huagang Hou^a, M. Lakshmi Kuppusamy^a, Thomas M. Drews^d, Anjali M. Prabhat^a, Sergey V. Petryakov^a, Eugene Demidenko^c, Philip E. Schaner^b, Jay C. Buckey^b, Aharon Blank^e, and Periannan Kuppusamy^{a,b,*}

^aDepartment of Radiology, Geisel School of Medicine, Dartmouth College, 1 Medical Center Drive, Lebanon, NH 03756, USA

^bDepartment of Medicine, Geisel School of Medicine, Dartmouth College, 1 Medical Center Drive, Lebanon, NH 03756, USA

^cDepartment of Biomedical Data Sciences, Geisel School of Medicine, Dartmouth College, 1 Medical Center Drive, Lebanon, NH 03756, USA

^dDepartment of Chemistry, University of Massachusetts, Amherst, MA 01003, USA

^eSchulich Faculty of Chemistry Technion - Israel Institute of Technology, Haifa 32000, Israel

Abstract

Purpose—Transcutaneous oxygen tension (TcpO₂) provides information about blood perfusion in the tissue immediately below the skin. These data are valuable in assessing wound healing problems, diagnosing peripheral vascular/arterial insufficiency, and predicting disease progression or the response to therapy. Currently, TcpO₂ is primarily measured using electrochemical skin sensors, which consume oxygen and are prone to calibration errors. The goal of the present study was to develop a reliable method for TcpO₂ measurement in human subjects.

Methods—We have developed a novel TcpO₂ oximetry based on electron paramagnetic resonance (EPR) principles with an oxygen-sensing skin adhesive film, named the superficial perfusion oxygen tension (SPOT) chip. The SPOT chip is a 3-mm diameter, 60-μm-thick circular film composed of a stable paramagnetic oxygen sensor. The chip is covered with an oxygen-barrier material on one side and secured on the skin by a medical adhesive transfer tape to ensure that only the oxygen that diffuses through the skin surface is measured. The method quantifies TcpO₂ through the linewidth of the EPR spectrum.

Results—Repeated measurements using a cohort of 10 healthy human subjects showed that the TcpO₂ measurements were robust, reliable and reproducible. The TcpO₂ values ranged from 7.8±0.8 to 22.0±1.0 mmHg in the volar forearm skin (N=29) and 8.1±0.3 to 23.4±1.3 mmHg in the foot (N=86).

*Corresponding author at: Geisel School of Medicine at Dartmouth, 1 Medical Center Drive, Rubin 601, Lebanon, NH 03756, USA. Tel: +1 603 653 3577. kuppu@dartmouth.edu.

Conclusions—The results demonstrated that the SPOT chip can measure $TcpO_2$ reliably and repeatedly under ambient conditions. The SPOT chip method could potentially be used to monitor $TcpO_2$ in the clinic.

Keywords

EPR; oximetry; transcutaneous; SPOT chip; TcOM; human subjects

1. Introduction

Since oxygen plays a critical role in many pathophysiological processes, quantifying oxygen concentrations in living systems is of paramount importance (1). But, a robust and reliable method for routine clinical use remains elusive. In the late 1980s, a computerized polarographic needle electrode system, called Eppendorf, was used extensively to assess tumor tissue oxygen levels in the clinic. Its utility was limited because it was invasive and unsuitable for repeated measurements (2). Currently, several techniques based on other principles exist, including fluorescence-quenching, phosphorescence-quenching, near-infrared, and magnetic resonance (3); however, their utility for routine clinical applications is limited. An ideal method for routine clinical use must be noninvasive, reliable, repeatable, and able to measure oxygen tension directly (partial pressure of oxygen, pO_2). In particular, the method must provide repeated measurements from the region of interest to monitor changes in oxygen tension over the observation or treatment period.

Transcutaneous oxygen monitoring (TcOM) is a noninvasive, clinically-approved method to obtain skin oxygen levels (4,5). The method is quantitative, and measures oxygen delivery to the skin from underlying tissue. It has been used to monitor transcutaneous oxygen tension ($TcpO_2$) in the skin, especially for premature infants, but also for adults in the intensive care setting (6). Physicians also use TcOM to determine whether or not adequate blood flow exists to heal lower extremity wounds, such as venous stasis or diabetic foot ulcers. Vascular surgeons use TcOM measurements to assess the need for amputation and determine the level where the amputation should take place. Unfortunately, the TcOM method has some significant limitations. The method is prone to calibration errors and the electrode consumes oxygen during measurement and is thus not capable of measuring low levels of oxygen that may be present in the skin under room temperature. To compensate for this, TcOM uses a heated metal electrode placed directly on the skin. During measurement, the skin beneath the electrode is heated up to 44°C , which dilates the vessels in the underlying tissue and increases oxygen permeability. Alternative methods based on optical sensors using fluorescence- (7), phosphorescence- (8–10) or luminescence-quenching (11,12) are under development to overcome the limitations associated with TcOM.

We are developing a novel oximetry method, based on electron paramagnetic resonance (EPR) using oxygen-sensing paramagnetic probes, for deep-tissue and superficial tissue measurements in the clinical setting (13,14). The principle of EPR oximetry is based on the paramagnetic characteristics of molecular oxygen, which in its ground state has two unpaired electrons, and undergoes spin exchange interaction with a paramagnetic EPR spin probe. This process is sensitive to the amount of oxygen present in the local environment,

with the relaxation rate of the spin probe increasing as a function of oxygen partial pressure (pO_2). The increased spin-spin relaxation rate results in increased line-broadening. The fact that the width of EPR resonance line correlates with oxygen tension has been used in a variety of biological applications (15–26). To perform repeated measurements of tissue pO_2 in humans, we have developed a novel class of implantable sensors using stable paramagnetic molecules or crystals embedded in biocompatible polymers (27–31). Recently, we have also started using EPR oximetry with an implantable sensor, called OxyChip, for measuring tumor oxygen levels in cancer patients (32). Initial clinical trial results indicate EPR oximetry could be a reliable and robust tool for clinical oximetry (33).

The goal of the present study was to develop and evaluate a novel EPR sensor and procedures for noninvasive monitoring of transcutaneous oxygen in human subjects. The method uses a paramagnetic chip that when placed on the skin is highly responsive to superficial perfusion oxygen tension (SPOT). The sensory element of the chip consists of microcrystals of lithium octa-*n*-butoxynaphthalocyanine (LiNc-BuO) embedded in polydimethylsiloxane (PDMS). The method is noninvasive, highly sensitive to low oxygen levels (hypoxia), and repeatable for long-term tissue oxygen monitoring. In this study, we have performed a preliminary evaluation of the SPOT chip's capabilities using a small ($N=10$) cohort of healthy human subjects. We present the results, which demonstrate the potential of this method for use in the clinic where routine monitoring of transcutaneous oxygen is required for prognosis and treatment of patients with perfusion-limited vascular diseases, chronic wounds, or skin malignancies.

2. Methods

2.1 Composition and fabrication of SPOT chip

The oxygen sensor (SPOT chip) is composed of two layers, an oxygen-sensing layer made of finely powdered microcrystals of LiNc-BuO embedded in polydimethylsiloxane (PDMS) polymer and an oxygen-barrier film (Fig. 1). The SPOT chip was fabricated by cast-molding polymerization and embedding processes using platinum silicone elastomer (MED-4210, medical-grade PDMS, from Factor II, Lakeside, AZ) and LiNc-BuO crystals (28). Microcrystals of LiNc-BuO were synthesized as reported by Pandian et al. (34), were suspended in distilled water, sonicated, and evaporated under ambient conditions to produce fine crystals of $< 2\text{-}\mu\text{m}$ size. The PDMS base and catalyst/cross-linker (supplied with the PDMS elastomer) were mixed in a 10:1 ratio, after which the LiNc-BuO crystals (40% by weight) were added. After the addition of $10\text{ }\mu\text{l}$ (about one drop) of platinum crosslinking accelerator (A-317), the heterogeneous dispersion/mixture was outgassed using a vacuum desiccator connected to a vacuum pump. Cast-molding of the product in the form of a thin sheet was made using two rectangular ($10 \times 5\text{ cm}$) transparent acrylic plates and a spacer film ($140\text{-}\mu\text{m}$ thick). An oxygen-barrier film (3M 3750P polypropylene tape, $79\text{-}\mu\text{m}$ thickness) was placed on one plate and degassed PDMS/LiNc-BuO mixture was transferred and evenly spread on the second plate. The two plates, with spacer films on the edges, were pressed together to produce a thin film cured at 70°C overnight. The SPOT chips were obtained by cutting the cured film into circular discs of 3-mm diameter using a Miltex medical biopsy dermal puncher. The thickness of the sensory part of the chip was about 60

µm. The size and shape of the SPOT can be easily adapted for different applications and measurement conditions, but we observed that this thickness and size of the chip was optimal for the skin measurements: the volume is small enough to enable relatively fast diffusion of oxygen into the sensor, but not too small to prevent enough EPR signal from being detected. The function of the oxygen-barrier backing film is to prevent oxygen diffusion from outside and to protect the integrity of the oxygen-sensing film from uneven contact with the skin.

2.2 Calibration of oxygen and temperature sensitivity

The oxygen sensitivity of the SPOT chip was measured and calibrated using an L-band EPR spectrometer as reported (32). Briefly, the chip was placed over a small opening of a custom-designed chamber with provisions for gas flow through it. A thermocouple (Omega HH23A type K Omega Engineering, Norwalk, CT) was placed on the chip and covered with an oxygen-barrier film (3M™ 1523 polyethylene tape) to prevent outside air from diffusing into the chip/chamber. A surface-loop resonator (35) was positioned above the chip for EPR measurements. For calibrations, pre-mixed gases with known O₂ concentration (Airgas USA, Cheshire, CT) were flushed into the calibration chamber until equilibrium is reached, usually in about 2–3 min. The EPR spectral width, calculated using a single-line Lorentzian model fitting, was used to construct the calibration curve. The effect of temperature on oxygen sensitivity (EPR line-width) was determined using preheated gas mixtures and temperature from the thermocouple located on the SPOT chip. The temperature calibrations were performed in the temperature range 22 to 45°C.

2.3 Protocol for measurement in human subjects

The study protocol was approved by Dartmouth's Committee for the Protection of Human Subjects (CPHS #28207). The study includes data obtained using 10 healthy volunteers (6 males and 4 females) between the ages of 18 and 63. The exclusion criteria, evaluated by screening questionnaire and interview with potential volunteers, were peripheral vascular disease, diabetes, hypertension, pregnancy, or medical implants that would be affected by a magnetic field. Multiple measurements were made on the same subjects with a new sensor applied each time and they were treated as independent measurements. Since the goal of the study was to establish the SPOT chip technology for transcutaneous pO₂ measurements, we did not consider gender, age, or race in our statistical analysis. Before EPR measurement, the site on the flexor side of forearm or between first and second metatarsal head of foot was cleaned with 70% isopropyl alcohol and the superficial horny scales were exfoliated by stripping 10 times with an adhesive tape (36,37). The SPOT chip was placed on the skin and secured with a single-sided, medically approved, oxygen-barrier tape (12.7-mm diameter; 3M™ 1523 polyethylene) coated with a pressure-sensitive acrylate adhesive (Fig. 1d). The adhesive tape was hypoallergenic, approved for use on human skin and easily peeled off without causing any discomfort or damage. The adhesive tape held the SPOT chip firmly to the skin, while also acting as another layer of insulation from ambient oxygen. We observed that the SPOT chip was insulated and intact (adhered) to the skin for more than 24 hours. In cases where the time course of TcPO₂ was followed, the measurements were started immediately after placement of the SPOT chip. In all other cases, the TcPO₂ measurements were made 60 – 120 min after placement of the chip to make sure the equilibrium value of

skin oxygenation would have been reached. In some cases, multiple chips were placed in different locations on the skin and measured individually. During measurement, a custom-built surface loop resonator with a flexible cable was placed over the chip and secured using an adhesive tape or fixation ring (Fig. 1e). The flexible cable reduces any artifacts due to voluntary or involuntary motion of the subject during the measurement.

2.4 EPR measurement in human subjects

All EPR measurements on human subjects were made using an L-band spectrometer (clinical scanner) developed by the EPR Center at Dartmouth College (38). Main components of the hardware (RF bridge, resonator) and software (online line-shape fitting) were customized and tuned to make *in vivo* EPR oximetry measurements. For measurements in the forearm, the subjects were asked to lay supine with forearm laid across the chest. For foot measurements, the subjects were seated on a chair with the foot down while the chair was raised and moved into the magnet. Subjects were allowed to rest for 5–10 min before the measurements started. For blood-flow constriction experiments, a phlebotomy tourniquet or blood pressure cuff was applied to the arm or leg where the SPOT chip was placed, proximal to the elbow or knee respectively. EPR measurements were made continuously for about 10 min with the tourniquet applied and for at least an additional 10 min after releasing the tourniquet. For hyperoxygenation experiments, following baseline measurements while the subject was breathing room air, the breathing gas was switched to 100% medical grade oxygen administered via a non-rebreather face mask at a flow rate of 15 L/min. EPR measurements were made continuously for 10 min during hyperoxygenation and for at least an additional 10 min after switching to room-air breathing. The EPR settings for the *in vivo* measurements were: microwave frequency ~1.2 GHz, incident RF power 0.6 mW, scan time 5 sec, scan range (dependent on the linewidth of the EPR signal), modulation frequency 21 kHz, modulation amplitude was maintained by the automated software at one-third of EPR line width to prevent over-modulation and artificial line broadening. The skin temperature at the SPOT chip location was monitored during EPR data acquisition. The average skin temperature in the foot of 5 subjects during 61 independent sessions was $34.3 \pm 1.1^\circ\text{C}$ before and $33.4 \pm 1.6^\circ\text{C}$ after EPR measurement suggesting that the low RF power used did not induce any significant ($P=0.30$) heating of the skin. No effort was made to maintain or modify the skin temperature in order to avoid any perturbation to the tissue perfusion. Also, skin humidity was not measured during the measurements.

Prior to placement on the skin, the chips were kept under ambient conditions, room temperature ($\sim 22^\circ\text{C}$) and room air (~ 160 mmHg); however, after placement of the chip on the skin both the temperature and pO_2 within the chip changes rapidly to reach equilibrium with the skin temperature and TcpO_2 . Since SPOT chip does not consume oxygen, the ambient oxygen trapped in the chip has to diffuse into the skin before establishing an equilibrium with the transcutaneous oxygen. This process, in the present design of SPOT chip, may take up to 60 min depending on the type of skin and conditions including humidity, temperature etc. In most of cases, the TcpO_2 values reported in this study were measured at least 60 min after placement of the chip. During the interim period, the subjects were free to move around or leave and come back for measurements. Throughout the

manuscript the use of the term ‘base value’ refers to the $TcpO_2$ measured after establishing equilibrium with the skin oxygen and not ambient oxygen.

2.5 TcOM measurements

The TcOM measurements were performed on the foot using TCM400 transcutaneous pO_2 monitoring system (Radiometer Medical ApS, Denmark) to compare with our SPOT chip technology. A single probe, with temperature set at 44°C , was used on the skin between the fourth and fifth metatarsal heads. An equilibrium period of about 30–40 min was taken before the reading was stabilized. The TcOM measurements were made always after EPR measurements to avoid the effect of heating (by TcOM) on the EPR data. Also, simultaneous measurements were not possible due to differences in the skin temperature in the two measurements and also because the TcOM probe head would interfere with the EPR magnetic field.

Statistical analyses

The statistical analyses were conducted using statistical package R, version 3.4.0 (39). To obtain the equilibrium $TcpO_2$ for the SPOT chip measurements the following three-parameter exponential regression model with the asymptote $f(t; a_1, a_2, a_3) = a_2 + (a_1 - a_2) \cdot \exp(-a_3 \cdot t)$ was used, where t measures time of the measurement (min). The parameters of the model have the following interpretation: a_1 estimates the $TcpO_2$ value at the start of the measurements ($t=0$), a_2 estimates the equilibrium $TcpO_2$ and a_3 estimates the rate at which $TcpO_2$ approaches the equilibrium level. The three parameters are estimated by the nonlinear least squares (40) using function **nls** in R. To compare the precision of TcOM and SPOT chip measurements the F-test for variances was used with the p-value computed using function **pf** in R. To test the homogeneity of SPOT chip measurements at different locations of the foot the one-way ANOVA was used with the p-value computed using the R function **anova**. To estimate the within and between patient variation of SPOT chip $TcpO_2$ measurements, the variance component model was employed (41). This model was estimated in R using function **lme** from the library **nlme**.

3. Results

3.1 Oxygen response and calibration of SPOT chip

The paramagnetic crystals of LiNc-BuO in the SPOT chip showed a single-line EPR spectrum with a linear relationship between spectral width and pO_2 in the range 0–160 mmHg (Fig. 2a). The spectrum also exhibited a linear dependence of width on temperature in the presence of oxygen (Fig. 2b). Since the skin temperature at the SPOT chip may vary widely depending on the skin and room temperature, it is necessary to calibrate the chip for the skin temperature. We measured the temperature-dependent width under different pO_2 to obtain calibration curves for temperatures in the range $22\text{--}45^\circ\text{C}$ (Fig. 2c). The linear nature of variation of the width with temperature and pO_2 makes it possible to use an appropriate sensitivity calibration for any temperature through the relationship: Sensitivity (mG/mmHg) = $16.0 - 0.161 \cdot T$, where T is temperature ($^\circ\text{C}$).

3.2 Time-course of TcpO₂ and its response to changes in underlying tissue

The time to reach equilibrium was determined by continuously monitoring the pO₂ after the SPOT chip was placed on the foot, between the first and second metatarsal heads, of healthy subjects. Fig. 3 shows a typical case where a TcpO₂ value of 22 mmHg was obtained in about 30 min after placement of the chip. While this might not be the equilibrium value yet, we determined whether the chip was responsive to changes in underlying tissue oxygenation. The blood flow to the foot was temporarily constricted using a blood-pressure cuff during which the TcpO₂ progressively declined to about 19 mmHg and returned to the pre-constriction value when the blood flow was restored. The results demonstrated the ability of the chip to respond to changes in underlying tissue oxygenation in the foot.

3.3 Response of TcpO₂ to oxygen deprivation and hyperoxygenation

The ability of SPOT chip to respond to dynamic changes in tissue oxygenation was determined in the forearm skin of healthy subjects. After reaching equilibrium TcpO₂, blood-flow to the forearm was temporarily constricted using a blood-pressure cuff. Fig. 4 shows the measurements in a single subject where the TcpO₂ declined from a steady-state base value of 27.1 ± 0.1 mmHg to 19.1 ± 0.1 mmHg in about 10 min of constriction and increased to 30.6 ± 0.2 mmHg after restoration of blood flow to the arm. This level was significantly higher than the base value, which is indicative of a reactive hyperemia. It was also observed that subsequent breathing of 100% oxygen, administered through a non-rebreather facemask, resulted in further increase of TcpO₂ to 38.0 ± 0.2 mmHg followed by a slow return toward base value. The results demonstrated the ability of the chip to respond to changes in tissue and transcutaneous oxygenation in the arm.

3.4 Kinetics of SPOT chip pO₂ towards establishing equilibrium with skin pO₂

When the SPOT chip is placed on the skin, the ambient oxygen trapped in the chip establishes a steep oxygen gradient across the skin resulting in the diffusion of oxygen into the skin before establishing equilibrium with the transcutaneous oxygen. We wanted to determine the kinetics of oxygen exchange and to know whether the information, e.g., rate of decay, would be a predictor of equilibrium TcpO₂. Immediately after the SPOT chips were applied to the skin of arm or foot of healthy subjects, the temporal changes in chip pO₂ was monitored continuously for up to 60 min. The pO₂ data obtained from the arm (Fig. 5a-c) and foot (Fig. 5d-f) showed an exponential decrease of pO₂ toward reaching an equilibrium (base) value. The rate constant of pO₂ drop and equilibrium TcpO₂ value were calculated using a single-exponential decay function with equilibrium model. The average rate constants (mean $\pm$ SD) obtained from arm (5 measurements on 3 subjects) and foot (9 measurements on 3 subjects) measurements were $10.5 \pm 2.4\%$ min⁻¹ and $6.7 \pm 0.9\%$ min⁻¹, respectively. The rate constants suggest that equilibrium is reached in about 44 min in the arm and 68 min in the foot. Overall, the results established that the base TcpO₂ values can be predicted from the decay data without having to wait until the equilibrium is reached.

3.5 Base values of TcpO₂ measured at multiple sites on the foot

We next determined whether the placement of the chip at different locations on the skin would have any effect on TcpO₂ values. The chips were placed at four different sites,

namely, the skin between the first and second and between the fourth and fifth metatarsal heads of the right and left foot of a healthy subject. As shown in Fig. 6, the TcpO₂ values measured from these four sites were comparable (Mean±SD: 10.9±4.3; 10.0±1.1; 11.3±2.4; 10.6±1.8 mmHg). The one-way ANOVA model and F-test were used to test whether the TcpO₂ measurements at four different spots on the foot were statistically different. The test gave a p-value = 0.932 indicating that they were not significantly different from each other. Overall, the results suggest that the skin oxygenation in the foot is fairly uniform and may not be sensitive to minor variations in chip placement for repeated measurements.

3.6 TcpO₂ values measured in the arm of healthy subjects

To evaluate the reliability of SPOT chip oximetry, we determined intra- and inter-person variability of TcpO₂ using a total of 28 measurements made in the flexor side of forearm of 5 healthy subjects over a period of several weeks. A new chip was applied at approximately the same location on the forearm for each measurement and subject. Fig. 7 shows the distribution TcpO₂ values within and among the subjects. The data exhibited a substantial variation of TcpO₂ between the subjects, from 7.8±0.8 mmHg to 22.0±1.0 mmHg for each subject. The mean value of all measurements was 12.8 mmHg, with a SD of the repeated measures within the group 3.7 mmHg and that between groups 5.4 mmHg. The results demonstrated the accuracy and reliability of transcutaneous oximetry by the SPOT chip.

3.7 TcpO₂ values measured in the foot of healthy subjects

We further determined intra- and inter-person variability of TcpO₂ in the foot of 8 healthy subjects using a total of 81 measurements over a period of several weeks. A new chip was applied at approximately the same location on the foot, between the first and second metatarsal heads for each measurement. The data showed a substantial variation of TcpO₂ between the subjects, from 8.1±0.3 mmHg to 23.4±1.3 mmHg, while the variation within each subject was less (Fig. 8). The mean value of all measurements was 15.3 mmHg, with a SD of repeated measures within the group 3.1 mmHg and that between groups 5.8 mmHg. The results established the ability of SPOT chip oximetry to provide reproducible and reliable TcpO₂ data.

3.8 Correlation of TcpO₂ data obtained using SPOT chip with TcOM oximetry

We next compared TcpO₂ data measured using SPOT chip at room temperature with TcOM, which requires heating of the skin while making measurements. We performed repeated TcpO₂ measurements in the foot of 4 healthy subjects over a period of 8 weeks. The SPOT chip measurements were performed with the skin at room temperature, while the TcOM measurements were done after heating the skin to 44°C at the location of the electrode. As expected, the TcpO₂ values obtained using TcOM were several folds higher (mean 68.0±2.1 mmHg; N=17) compared to SPOT chip (mean 11.0±1.3 mmHg; N=17) likely due to the skin heating which alters both cutaneous blood flow and oxygen permeability. Fig. 9a shows a comparison of the two groups that indicates a weak correlation ($r = 0.40$) between the data sets obtained from all subjects. On the other hand, as shown in Fig. 9b, the pO₂ values obtained from a single subject showed a better correlation ($r = 0.59$) between data sets. The Bland-Altman technique to compare SPOT chip with TcOM pO₂ measurements could not be used because this method requires that the compared samples have the same variance, which

is not true in our case. Since the mean values of $TcpO_2$ for TcOM are positively biased due to heating we compared the precision of the two methods using standard deviations, 8.6 mmHg for TcOM and 5.5 mmHg for the SPOT chip. The one-sided F-test for variances was used for statistical comparison (42), which gave a p value of 0.043. According to traditional statistical threshold for the type I error 5% we reject the null hypothesis that the two methods have the same variance/precision.

4. Discussion

In the present work, we evaluated the SPOT chip technology for measuring transcutaneous oxygen tension in human subjects. The results demonstrated that the SPOT chip method is robust, highly sensitive to low levels of oxygen such as those that occur in pathophysiological conditions, and that the pO_2 measurements can be performed at room temperature and at the physiological skin temperature. The SPOT chip technology is a noninvasive method for measuring tissue oxygenation. It can provide clinicians with real-time information about tissue oxygen levels in wounded or injured tissue. This technology could potentially be used to decrease mortality in critically ill patients. Giving surgeons quantitative measurements on levels of tissue oxygenation can help guide their debridement decisions to avoid taking too much tissue (which may increase the difficulty of reconstruction or compromise function of the remaining tissue) or avoid taking too little, which may result in tissue necrosis and infection. It may reduce the number of times a patient must be taken to the operating room for a surgical debridement and decrease the incidence of surgical site infections (SSIs) by reducing the likelihood of leaving behind non-viable tissue. It can be used to monitor the viability of tissue flaps used to close defects due to trauma, cancer or congenital causes as well as to identify early changes in oxygenation to recognize a compromised flap. An earlier return to the operating room to revise compromised flaps will result in an increased flap salvage rate. The technique could also potentially be used to track changes in vascular supply, such as due to hyperbaric oxygen or angiogenic agents.

A notable feature of the SPOT chip is its linear and reliable calibration for use at a range of temperatures. We have previously shown that embedding LiNc-BuO crystals in PDMS provides a robust formulation where the calibration of the chip is stable under conditions of high-temperature sterilization and exposures to ionizing radiation (32). We have also shown that the chip implanted in the rat gastrocnemius muscle was capable of providing repeated and real-time measurements of tissue oxygenation for up to one year without any loss of EPR or oxygen sensitivity. Further, no inflammatory or adverse reaction at or around the implantation area was observed thereby establishing its biocompatibility and safety (32). The effect of temperature on the oxygen sensitivity of SPOT chip is anticipated on the basis that the oxygen-induced exchange broadening occurs exclusively in the gas phase, that is, by diffusion of molecular oxygen into the empty tunnels in the crystal (43). The temperature studies show a predictable linear behavior, enabling a reliable calibration at measurement temperatures. The EPR detection sensitivity depends on the spin composition of the chip, which is 40% by weight in our case. We observed that this is the highest possible density that we can use reliably for the fabrication of the chip. The oxygen sensitivity, however, is determined from the EPR width and hence is not influenced by the spin content of the chip.

The preliminary data collected using SPOT chip establish that the method is reliable and reproducible for real-time measurements in human subjects. The mean TcpO₂ data collected from arm and foot measurements demonstrate that there is less variation within each subject, while there is a substantial distribution among the subjects, suggesting that the measured values may be an accurate representation of skin perfusion in individual subjects and not due to measurement uncertainty. The reliability of the measurements is also evident from the correlation between the SPOT chip and TcOM data, despite the fact that these data were collected from a small set of subjects and at two different skin temperatures, ~33°C for SPOT chip and 44°C for TcOM. Additional measurements with increased number of subjects are needed to establish the relationship between these two methods.

The temperature of the skin has a profound effect on the transcutaneous oxygen value. An increase in temperature can increase blood perfusion in the underlying tissue (hyperemia), shift the oxyhemoglobin dissociation curve, and melt lipids in the intact stratum corneum thereby enhancing the diffusion of oxygen across the skin (37,44). Electrochemical methods, such as TcOM, take advantage of this effect by increasing the measurement temperature to 40°C and above. Skin oxygen levels at room temperature (where skin temperature is approximately 33°C), has been measured by optical methods, but it is too small for TcOM due to consumption of oxygen by the electrode during measurement. As a result, TcOM measurements show 0 mmHg at normal skin temperature (45) and heating is an obligatory requirement for TcOM operation. Heating produces an elevated TcpO₂ which is often postulated to as representative of underlying tissue perfusion. But, the true pO₂ on the skin surface can only be measured by methods that do not consume oxygen during measurement (46). Optical methods based on fluorescence- (7), phosphorescence- (8–10) or luminescence-quenching (11,12) and EPR (32,47), including SPOT chip, do not use oxygen and hence are capable of reporting true skin pO₂ at ambient or physiological temperatures. For example, an oxygen optode device based on fluorescence lifetime imaging (FLIM), reported an average of ~20 mmHg of TcpO₂ in the medial lower leg of human subjects (N=5) measured by heating the skin to 37°C (48). Another optical device, based on luminescence lifetime imaging (LLI), in the volar forearm skin heated to 40°C reported a TcpO₂ of 66.1±7.7 mmHg (11). Another study using the LLI method on chronic wounds reported ~37 mmHg measured at room temperature (12); however, it is questionable whether the measurement on the wound surface would fit the definition of TcpO₂ as the measurement was not on the skin. A TcOM (electrode) device used at 37°C in the abdominal skin (12 subjects) and ankle (20 subjects) reported an average TcpO₂ value of 8.7±1.1 mmHg and 10.8±9.6 mmHg, respectively, which might have underestimated the true TcpO₂ due to its consumption of oxygen (37). Unlike the optical and TcOM methods, the SPOT chip has a higher sensitivity at lower levels of oxygen due to the fact that EPR sensitivity of detection is inversely proportional to pO₂, as shown in Fig. 1a. Overall, the SPOT chip has the unique capability to measure TcpO₂ at ambient temperature, thus capable of reporting TcpO₂ under unperturbed conditions.

The development and validation of SPOT chip technology would provide a means by which to collect tissue oxygen data noninvasively in a number of clinical situations, including some for which TcOM could not be used. For wound-healing applications, TcOM sensors are placed around the wound, but never inside the wound bed. Because SPOT chips are

composed of biocompatible materials and do not actively heat the tissue, they could be placed within the wound bed to obtain data from this region of tissue, which is a key advantage over the TcOM method. Similarly, for plastic surgery applications, SPOT chips could be placed on or within the underlying areas where tissue flaps are placed for reconstructive procedures. The SPOT technology could serve to decrease the incidence of surgical site infections, which will also result in a decrease in mortality and length of hospital stay. It also has the potential to decrease mortality rates for patients in the ICU. It can help guide the treatment of patients with chronic open wounds and make reconstructive surgery more successful by providing better flap monitoring. It may also be used for ventilated patients as a marker for arterial pO_2 , saving on taking blood samples. It could be used to track changes in vascular perfusion over time due to interventions such as stents, angioplasties, angiogenic agents, stem-cell therapy, or hyperbaric oxygen treatment. Despite the advantages of the SPOT chip method for making surface or transcutaneous pO_2 measurements, translation of the technology for routine clinical use will require additional efforts including modifying the currently accepted clinical practice of using TcOM data. Most importantly, since the SPOT chip will be used at ambient temperatures (without heating of the skin) the interpretation of the pO_2 data as to hypoxia or normoxia would require rescaling of the hypoxia threshold for proper diagnostic or prognostic decision making by the clinician.

5. Conclusions

We have performed a preliminary evaluation and testing of SPOT chip technology for measurement of transcutaneous oxygen tension in human subjects. The method is noninvasive and highly sensitive to low oxygen levels, such as those that occur in pathophysiological conditions. Initial measurements in human subjects have demonstrated that the SPOT chip can be used to measure transcutaneous pO_2 under ambient conditions. Overall, the study established the SPOT chip as a promising superficial tissue oximetry probe with potential applications in the clinic.

Acknowledgements

This study was supported by National Institutes of Health (NIH) grants R01 EB004031–12, R21 EB 022247–02, and R21 EB016189–02.

References

1. Carreau A, El Hafny-Rahbi B, Matejuk A, Grillon C, Kieda C. Why is the partial oxygen pressure of human tissues a crucial parameter? Small molecules and hypoxia. *J Cell Mol Med* 2011;15(6): 1239-1253. [PubMed: 21251211]
2. Vaupel P, Hockel M, Mayer A. Detection and characterization of tumor hypoxia using pO_2 histography. *Antioxid Redox Signal* 2007;9(8):1221-1235. [PubMed: 17536958]
3. Bickler P, Feiner J, Rollins M, Meng L. Tissue Oximetry and Clinical Outcomes. *Anesth Analg* 2017;124(1):72-82. [PubMed: 27308951]
4. Huch R, Lubbers DW, Huch A. Quantitative continuous measurement of partial oxygen pressure on the skin of adults and newborn babies. *Pflügers Arch ges Physiol* 1972;337:185-198. [PubMed: 4677157]

5. Huch R, Huch A, Lubbers DW. Transcutaneous measurement of blood Po₂ (tcPo₂) — Method and application in perinatal medicine. *Journal of Perinatal Medicine* 1973;1(3):183-191. [PubMed: 4806572]
6. Kaur S, Pawar M, Banerjee N, Garg R. Evaluation of the efficacy of hyperbaric oxygen therapy in the management of chronic nonhealing ulcer and role of periwound transcutaneous oximetry as a predictor of wound healing response: A randomized prospective controlled trial. *J Anaesthesiol Clin Pharmacol* 2012;28(1):70-75. [PubMed: 22345950]
7. Stucker M, Struk A, Altmeyer P, Herde M, Baumgartl H, Lubbers DW. The cutaneous uptake of atmospheric oxygen contributes significantly to the oxygen supply of human dermis and epidermis. *J Physiol* 2002;538(Pt 3):985-994. [PubMed: 11826181]
8. Geis S, Babilas P, Schreml S, Angele P, Nerlich M, Jung EM, Prantl L. Transcutaneous pO₂ measurement during tourniquet-induced venous occlusion using dynamic phosphorescence imaging. *Clin Hemorheol Microcirc* 2008;40(4):249-258. [PubMed: 19126987]
9. Li Z, Navarro-Alvarez N, Keeley EJ, Nowell NH, Goncalves BMM, Huang CA, Evans CL. Non-invasive monitoring of skin inflammation using an oxygen-sensing paint-on bandage. *Biomed Opt Express* 2017;8(10):4640-4651. [PubMed: 29082091]
10. Li Z, Roussakis E, Koolen PG, Ibrahim AMS, Kim K, Rose LF, Wu J, Nichols AJ, Baek Y, Birngruber R, Apiou-Sbirlea G, Matyal R, Huang T, Chan R, Lin SJ, Evans CL. Non-invasive transdermal two-dimensional mapping of cutaneous oxygenation with a rapid-drying liquid bandage. *Biomed Opt Express* 2014;5(11):3748-3764. [PubMed: 25426308]
11. Babilas P, Lamby P, Prantl L, Schreml S, Jung EM, Liebsch G, Wolfbeis OS, Landthaler M, Szeimies RM, Abels C. Transcutaneous pO₂ imaging during tourniquet-induced forearm ischemia using planar optical oxygen sensors. *Skin Res Technol* 2008;14(3):304-311. [PubMed: 19159376]
12. Schreml S, Meier RJ, Kirschbaum M, Kong SC, Gehmert S, Felthaus O, Kuchler S, Sharpe JR, Woltje K, Weiss KT, Albert M, Seidl U, Schroder J, Morsczech C, Prantl L, Duschl C, Pedersen SF, Gosau M, Berneburg M, Wolfbeis OS, Landthaler M, Babilas P. Luminescent dual sensors reveal extracellular pH-gradients and hypoxia on chronic wounds that disrupt epidermal repair. *Theranostics* 2014;4(7):721-735. [PubMed: 24883122]
13. Swartz HM, Hou H, Khan N, Jarvis LA, Chen EY, Williams BB, Kuppusamy P. Advances in probes and methods for clinical EPR oximetry. *Adv Exp Med Biol* 2014;812:73-79. [PubMed: 24729217]
14. Swartz HM, Williams BB, Zaki BI, Hartford AC, Jarvis LA, Chen EY, Comi RJ, Ernstoff MS, Hou H, Khan N, Swarts SG, Flood AB, Kuppusamy P. Clinical EPR: unique opportunities and some challenges. *Acad Radiol* 2014;21(2):197-206. [PubMed: 24439333]
15. Takakusagi Y, Kishimoto S, Naz S, Matsumoto S, Saito K, Hart CP, Mitchell JB, Krishna MC. Radiotherapy Synergizes with the Hypoxia-Activated Prodrug Evofosfamide: In Vitro and In Vivo Studies. *Antioxid Redox Signal* 2018;28(2):131-140. [PubMed: 28741367]
16. Kishimoto S, Matsumoto KI, Saito K, Enomoto A, Matsumoto S, Mitchell JB, Devasahayam N, Krishna MC. Pulsed Electron Paramagnetic Resonance Imaging: Applications in the Studies of Tumor Physiology. *Antioxid Redox Signal* 2018.
17. Desmet CM, Vandermeulen G, Bouzin C, Lam MC, Preat V, Leveque P, Gallez B. EPR monitoring of wound oxygenation as a biomarker of response to gene therapy encoding hCAP-18/LL37 peptide. *Magn Reson Med* 2018;79(6):3267-3273. [PubMed: 28983954]
18. De Preter G, Deriemaeker C, Danhier P, Brisson L, Cao Pham TT, Gregoire V, Jordan BF, Sonveaux P, Gallez B. A Fast Hydrogen Sulfide-Releasing Donor Increases the Tumor Response to Radiotherapy. *Mol Cancer Ther* 2016;15(1):154-161. [PubMed: 26682572]
19. Colliez F, Fruytier AC, Magat J, Neveu MA, Cani PD, Gallez B, Jordan BF. Monitoring Combretastatin A4-induced tumor hypoxia and hemodynamic changes using endogenous MR contrast and DCE-MRI. *Magn Reson Med* 2016;75(2):866-872. [PubMed: 25765253]
20. Khan N, Hou H, Eskey CJ, Moodie K, Gohain S, Du G, Hodge S, Culp WC, Kuppusamy P, Swartz HM. Deep-tissue oxygen monitoring in the brain of rabbits for stroke research. *Stroke* 2015;46(3):e62-66. [PubMed: 25613304]
21. Mohan IK, Khan M, Wisel S, Selvendiran K, Sridhar A, Carnes CA, Bognar B, Kalai T, Hideg K, Kuppusamy P. Cardioprotection by HO-4038, a novel verapamil derivative, targeted against

- ischemia and reperfusion-mediated acute myocardial infarction. *Am J Physiol Heart Circ Physiol* 2009;296(1):H140-151. [PubMed: 18978191]
22. Khan M, Mohan IK, Kutala VK, Kotha SR, Parinandi NL, Hamlin RL, Kuppusamy P. Sulfaphenazole protects heart against ischemia-reperfusion injury and cardiac dysfunction by overexpression of iNOS, leading to enhancement of nitric oxide bioavailability and tissue oxygenation. *Antioxid Redox Signal* 2009;11(4):725-738. [PubMed: 18855521]
 23. Khan M, Kutala VK, Vikram DS, Wisel S, Chacko SM, Kuppusamy ML, Mohan IK, Zweier JL, Kwiatkowski P, Kuppusamy P. Skeletal myoblasts transplanted in the ischemic myocardium enhance in situ oxygenation and recovery of contractile function. *Am J Physiol Heart Circ Physiol* 2007;293(4):H2129-2139. [PubMed: 17660391]
 24. Elas M, Ahn KH, Parasca A, Barth ED, Lee D, Haney C, Halpern HJ. Electron paramagnetic resonance oxygen images correlate spatially and quantitatively with Oxylite oxygen measurements. *Clin Cancer Res* 2006;12(14 Pt 1):4209-4217. [PubMed: 16857793]
 25. Ilangovan G, Liebgott T, Kutala VK, Petryakov S, Zweier JL, Kuppusamy P. EPR oximetry in the beating heart: myocardial oxygen consumption rate as an index of postischemic recovery. *Magn Reson Med* 2004;51(4):835-842. [PubMed: 15065258]
 26. Ilangovan G, Bratasz A, Li H, Schmalbrock P, Zweier JL, Kuppusamy P. In vivo measurement and imaging of tumor oxygenation using coembedded paramagnetic particulates. *Magn Reson Med* 2004;52(3):650-657. [PubMed: 15334586]
 27. Meenakshisundaram G, Eteshola E, Blank A, Lee SC, Kuppusamy P. A molecular paramagnetic spin-doped biopolymeric oxygen sensor. *Biosens Bioelectron* 2010;25(10):2283-2289. [PubMed: 20371170]
 28. Meenakshisundaram G, Eteshola E, Pandian RP, Bratasz A, Lee SC, Kuppusamy P. Fabrication and physical evaluation of a polymer-encapsulated paramagnetic probe for biomedical oximetry. *Biomed Microdevices* 2009;11(4):773-782. [PubMed: 19291409]
 29. Meenakshisundaram G, Eteshola E, Pandian RP, Bratasz A, Selvendiran K, Lee SC, Krishna MC, Swartz HM, Kuppusamy P. Oxygen sensitivity and biocompatibility of an implantable paramagnetic probe for repeated measurements of tissue oxygenation. *Biomed Microdevices* 2009;11(4):817-826. [PubMed: 19319683]
 30. Meenakshisundaram G, Pandian RP, Eteshola E, Lee SC, Kuppusamy P. A paramagnetic implant containing lithium naphthalocyanine microcrystals for high-resolution biological oximetry. *J Magn Reson* 2010;203(1):185-189. [PubMed: 20006529]
 31. Pandian RP, Meenakshisundaram G, Bratasz A, Eteshola E, Lee SC, Kuppusamy P. An implantable Teflon chip holding lithium naphthalocyanine microcrystals for secure, safe, and repeated measurements of pO₂ in tissues. *Biomed Microdevices* 2010;12(3):381-387. [PubMed: 20058084]
 32. Hou H, Khan N, Gohain S, Kuppusamy ML, Kuppusamy P. Pre-clinical evaluation of OxyChip for long-term EPR oximetry. *Biomed Microdevices* 2018;20(2):29. [PubMed: 29549438]
 33. Jarvis LA, Williams BB, Schaner PE, Chen EY, Angeles CV, Hou H, Schreiber W, Wood VA, Flood AB, Swartz HM, Kuppusamy P. Phase 1 Clinical Trial of OxyChip, an Implantable Absolute pO₂ Sensor for Tumor Oximetry. *Int J Radiat Oncol* 2016;96(2):S109-S110.
 34. Pandian RP, Parinandi NL, Ilangovan G, Zweier JL, Kuppusamy P. Novel particulate spin probe for targeted determination of oxygen in cells and tissues. *Free Radic Biol Med* 2003;35(9):1138-1148. [PubMed: 14572616]
 35. Salikhov I, Hirata H, Walczak T, Swartz HM. An improved external loop resonator for in vivo L-band EPR spectroscopy. *J Magn Reson* 2003;164(1):54-59. [PubMed: 12932455]
 36. Sejrson P. Epidermal diffusion barrier to ¹³³Xe in man and studies of clearance of ¹³³Xe by sweat. *J Appl Physiol* 1968;24(2):211-216. [PubMed: 5637684]
 37. Stucker M, Struk PA, Hoffmann K, Schulze L, Rochling A, Lubbers DW. The transepidermal oxygen flux from the environment is in balance with the capillary oxygen supply. *J Invest Dermatol* 2000;114(3):533-540. [PubMed: 10692114]
 38. Salikhov I, Walczak T, Lesniewski P, Khan N, Iwasaki A, Comi R, Buckey J, Swartz HM. EPR spectrometer for clinical applications. *J Magn Reson* 2005;54:1317-1320.
 39. Team RC. R: A language and environment for statistical computing. R Foundation for Statistical Computing Vienna, Austria 2014.

40. Bates DM, Watts DG. Nonlinear Regression and Its Applications. New York: Wiley; 1988.
41. Demidenko E Mixed Models: Theory and Applications with R. Hoboken: Wiley; 2013.
42. Snedecor GW, Cochran WG. Statistical Methods. Iowa: Iowa State University Press; 1989.
43. Pandian RP, Kim YI, Woodward PM, Zweier JL, Manoharan PT, Kuppusamy P. The open molecular framework of paramagnetic lithium octabutoxynaphthalocyanine: implications for the detection of oxygen and nitric oxide using EPR spectroscopy. *Mater J Chem* 2006;16(36): 3609-3618.
44. Van Duzee BF. Thermal analysis of human stratum corneum. *J Invest Dermatol* 1975;65:404-408. [PubMed: 1176792]
45. Evans NTS, Naylor PFD. The systemic oxygen supply to the surface of the human skin. *Respir Physiol* 1967;3:21-27. [PubMed: 6059101]
46. Lubbers DW. Theoretical basis of the transcutaneous blood gas measurements. *Crit Care Med* 1981;9:721-733. [PubMed: 7285610]
47. Ahmad R, Kuppusamy P. Theory, instrumentation, and applications of electron paramagnetic resonance oximetry. *Chem Rev* 2010;110(5):3212-3236. [PubMed: 20218670]
48. Stucker M, Schulze L, Pott G, Hartmann P, Lubbers DW, Rochling A, Altmeyer P. FLIM of luminescent oxygen sensors: clinical applications and results. *Sensors and Actuators B* 1998;51:171-175.

Fig. 1. Design and placement of SPOT chip for transcutaneous oximetry.

(a-c) Cross-sectional and exploded views of SPOT chip showing the components and assembly. The chip is made up of two layers of films bonded together; a circular film of 3-mm diameter and 60- μm thickness containing fine crystals of LiNc-BuO in PDMS and an oxygen-barrier film (79- μm thickness) on top. The chip is attached to the skin using an adhesive film (12.7-mm diameter and 90- μm thickness), which is also impermeable to oxygen. Overall, the SPOT chip placed on the skin will have two layers oxygen-barrier films to insulate the chip from ambient oxygen. (d) Photograph showing the placement of SPOT

chip on a volunteer's forearm skin. (c) Photograph showing the attachment of a flexible loop resonator over the SPOT chip for EPR measurements.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Fig. 2. Oxygen response and calibration of SPOT chip for transcutaneous oximetry.

(a) Effect of partial pressure of oxygen (pO_2) on the EPR spectrum of a SPOT chip measured at room temperature, $\sim 22^{\circ}C$. The spectral width shows a linear dependence on pO_2 in the range 0 to 160 mmHg (inset). (b) Effect of temperature on the EPR width of SPOT chip exposed to different pO_2 . The solid lines represent a linear fit to the respective data set. The data show a linear dependence of width on temperature in all cases. The temperature has no effect in the absence of oxygen (i.e., $pO_2 = 0$ mmHg). (c) Expression of width as a function of pO_2 (calibration) at four selected temperatures, namely 22 $^{\circ}C$, 33 $^{\circ}C$,

37°C, and 44°C calculated using the data in **(b)**. The calibration data are representative of one of three chips measured. The results show a decrease in oxygen sensitivity of SPOT chip with increase of temperature.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Fig. 3. Time-course of $TcpO_2$ and its response to changes in underlying tissue oxygenation in the foot.

A SPOT chip, kept in room air, was placed on the foot skin of a healthy subject between the first and second metatarsal heads. The $TcpO_2$ value in the chip was measured continuously until a base value (22.5 mmHg), indicated by dotted line, was reached in about 30 min after placement of the chip. Each data point in the curve represents the pO_2 value from a single 5-sec scan and the error bars represent fitting error (SD). At this time, blood flow to the foot was constricted using blood-pressure cuff and maintained for about 10 min and then released, as indicated in the chart. The $TcpO_2$ declined upon constriction to 19.0 mmHg and quickly returned to the base value after restoration of blood flow (inset). The results demonstrate the ability of the chip to respond to changes in tissue and transcutaneous oxygenation in the foot.

Fig. 4. Response of TcpO₂ to changes in underlying tissue oxygenation in the arm.

A SPOT chip, kept in room air, was placed on the flexor forearm skin of a healthy subject. After reaching a steady-state base value (TcpO₂, 27.1 mmHg, dotted line), blood flow to the arm was constricted using blood-pressure cuff and maintained for a brief period and then released, as indicated by arrows. During the constriction period the TcpO₂ declined to 19.1 mmHg with a time constant of 6.4 min. Upon restoration of blood flow, the TcpO₂ increased to 30.6 mmHg with a time constant of 6.6 min. At this time, breathing of 100% oxygen, administered through a non-rebreather facemask during the period marked by arrows, resulted in further increase of TcpO₂ to 38.0 mmHg followed by a slow return towards base value. The bar graph (inset) shows the peak TcpO₂ values (Mean ± SD) obtained by averaging of 10 measurements at the end of each manipulation of flow/oxygen. The results demonstrate the ability of the chip to respond to changes in tissue and transcutaneous oxygenation in the arm.

Fig. 5. Kinetics of SPOT chip pO_2 toward establishing equilibrium with skin pO_2 .

SPOT chips, kept in room air, were placed on the skin of arm or foot of healthy subjects. Following a delay of 3–5 min while the subject was prepared for EPR measurements, the temporal changes in chip pO_2 was monitored continuously for up to 60 min. The pO_2 data from the arm (a-c) and foot (d-f) show an exponential decrease of pO_2 toward reaching an equilibrium (base) value. The decay rates (inverse of first order rate constant) and equilibrium $TcpO_2$ values, calculated using a single-exponential decay with equilibrium model, are shown in the respective panels. The quality of fit suggest that the base $TcpO_2$ values can be predicted from the decay data without having to wait until the equilibrium is reached.

Fig. 6. TcpO₂ values measured at multiple sites on the foot.

TcpO₂ values were measured at two different spots in each foot of a healthy subject. Four chips were placed on the skin between the first and second metatarsal heads of right (R2) and left (L1) foot and the fourth and fifth metatarsal heads of right (R1) and left (L2) foot as shown in the inset photo. The base pO₂ values from the 4 chips were measured sequentially. The measurements were repeated 4 times on the same subject over a period of 2 weeks using new chips each time. The data, which represent Mean ± SD (N=4), did not show any statistically significant difference ($p=0.932$) among the 4 sites measured.

Fig. 7. Summary of TcpO₂ values measured in the arm of healthy subjects.

A total of 29 measurements were performed in the flexor forearm of 5 healthy subjects. The measurements were performed multiple times over a period of 24 weeks, by placing a new SPOT chip each time. (a) TcpO₂ values in subjects 1–5 show the distribution within and among the subjects. The median value is indicated by the horizontal bar. (b) The average TcpO₂ value (Mean ± SD) for each subject. Overall, the results from the arm demonstrate a substantial variation of TcpO₂ among the subjects measured.

Fig. 8. Summary of TcpO₂ values measured in the foot of healthy subjects.

A total of 86 measurements were performed in the foot of 8 healthy subjects. The measurements were performed multiple times over a period of 24 weeks, by placing a new SPOT chip each time. (a) TcpO₂ values in subjects 1–8 show the distribution within and among the subjects. The median value is indicated by the horizontal bar. (b) The average TcpO₂ value (Mean ± SD) for each subject. Overall, the results from the foot demonstrate a substantial variation of TcpO₂ among the subjects measured.

Fig. 9. Correlation of TcpO₂ data obtained by SPOT chip with TcOM oximetry.

SPOT chip and TcOM measurements of TcpO₂ were performed in the foot of 4 subjects for a total of 16 times. Of note, SPOT chip measurements were done at room temperature with the skin temperature at $33.5 \pm 1.0^\circ\text{C}$, while the TcOM measurements were done, by requirement, heating the skin to 44°C . Collectively, the TcpO₂ data obtained by the TcOM oximetry were several folds higher compared to the SPOT chip. **(a)** Pooled data from all subjects suggest a weak correlation ($r = 0.39$) between the sets. **(b)** Data obtained from a single subject show an improved (moderate) correlation ($r = 0.58$).