Clinical and Statistical Considerations when Assessing Oxygen Levels in Tumors: Illustrative Results from Clinical EPR Oximetry Studies

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Abstract The success of treatment for malignancies, especially those undergoing radiation therapy or chemotherapy, has long been recognized to depend on the degree of hypoxia in the tumor. In addition to the prognostic value of knowing the tumor's initial level of hypoxia, assessing the tumor oxygenation during standard therapy or oxygen-related treatments (such as breathing oxygen-enriched gas mixtures or taking drugs that can increase oxygen supply to tissues) can provide valuable data to improve the efficacy of treatments. A series of early clinical studies of tumors in humans are ongoing at Dartmouth and Emory using electron paramagnetic resonance (EPR) oximetry to assess tumor oxygenation, initially and over time during either natural disease progression or treatment. This approach has the potential for reaching the long-sought goal of enhancing the effectiveness of cancer therapy.

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In order to effectively reach this goal, we consider the validity of the practical and statistical assumptions when interpreting the measurements made *in vivo* for patients undergoing treatment for cancer.

Keywords EPR oximetry · Measures of oxygen · Clinical applications

1 Introduction

Hypoxia in malignant tumors has been well established to be associated with poor prognosis due both to its association with more aggressive growth and with greater resistance to therapy, including radiation and chemotherapy [1]. Hypoxia's enhancement of tumor resistance is generally thought to be due to the physical/chemical effects that negate the process by which therapeutic interventions work to destroy tumors. For example, the sustainability of damage to malignant cells caused by ionizing radiation depends on the immediate presence of molecular oxygen (O_2) to block the tumor cell's ability to repair the damage [2, 3]. There is evidence of increased survival and reduced recurrence of tumors that are reasonably well-oxygenated, in comparison to those that are hypoxic at the time of treatment [1, 4–8]. Consequently, clinical studies have attempted to increase the O_2 available in tumor cells coincident with active therapy, with the goal of increasing the therapeutic effect. However, when various methods to increase the O_2 in the tumors have been used in clinical trials, the results have been surprisingly weak [9, 10] - one reason why hyperoxygenation during therapy has yet to be adopted into clinical practice.

Why doesn't adding oxygen to the microenvironment of tumor cells appear to improve outcomes? One explanation is that only hypoxic tumors may benefit from the attempt to increase the oxygen delivery, as shown in the ARCON study [11]. There is another large body of literature looking at the partial pressure of oxygen (pO_2) in tissues that provides strong evidence of the heterogeneity of pO_2 in tissues. Heterogeneity exists in normal tissues, i.e., there are large differences in 'average' pO_2 or concentration of oxygen by types of tissue [12, 13]. More to the point about tumors, there is a large variation within a given tumor at a given point in time [14–16]. To add to this complexity, other work has demonstrated the continual dynamics of the amount of O_2 present in tissues as a result of the ever-changing gradients of O_2 that evolve in the microenvironment of cells [17]. Two main processes drive this dynamic: metabolic consumption of oxygen and the resulting diffusion of O_2 into the tissues in response to the presence of lower pO_2. Finally, pathological processes introduce variability relating to *chronic* vs *acute* hypoxia [18, 19]) and serve to up-regulate *or* down-regulate these effects, depending on the microenvironment [20].

Nevertheless, to establish clinically meaningful diagnostic measurements of oxygen in relevant tissue and thereby improve outcomes, clinicians and researchers continue to try to unravel these dynamic and complex relationships between hypoxia and outcomes. Indeed, the potential for oximetry to become a useful diagnostic tool

to improve clinical outcomes is expanding beyond tumors. For example, new studies are underway to try to understand the role of hypoxia in causing some of the debilitating side effects of cancer therapy such as radiation-induced fibrosis [21, 22] or chemotherapy-induced peripheral neuropathy or peripheral vascular disease [23, 24]. Likewise, studies are being designed to examine other pathologies related to hypoxia, e.g., peripheral vascular problems associated with diabetes or poor healing following surgery, with the ultimate goal of improving care, especially by personalizing the knowledge of each patient's hypoxic tissue and his/her responsiveness to hyperoxygenation to alter the outcomes.

For all these reasons, it is important to understand the promise and pitfalls of currently used methods to measure O_2 in tissues. Swartz et al. in a companion article [25] focus on the biological and clinical implications of measurement techniques, using electron paramagnetic resonance (EPR) oximetry as an illustrative technique. Here, we focus on the practical qualities and limitations of these same EPR techniques, especially those based on carbon particulates, and bear in mind the ability to carry out oximetry in a usual healthcare setting and a typical clinical workflow. We discuss the strengths and weaknesses of several statistical models to summarize EPR data into indicators of hypoxia or a response to hyperoxygenation.

2 Materials and Methods and What they Illustrate

In vivo EPR oximetry has a long history and is detailed elsewhere [26–29]. Briefly, the instrument used in this paper is an identical clone of the instrument developed and used at Dartmouth Medical School. The major difference between the ongoing studies is the type of oxygen sensor used, each of which requires being implanted or injected into the tissue of interest (see Table 1). After a sensor is implanted in the tissue (and after a suitable time for healing the minor wounds associated with this invasive step), EPR measurements can be made easily via a small sensor loop placed externally on the surface above the sensor. These non-perturbing EPR measurements can be repeated as long as the sensor remains in the body.

Table 1 compares and contrasts basic features for the three types of EPR sensors: oxygen-sensitive carbon particulates [30–32], crystals embedded in a polymer [33–35], and the implantable resonator [36]. The first two sensors are used in ongoing studies in humans; the implantable resonator is currently only in preclinical use.

The principal sensor focused on is composed of carbon particles suspended in a pharmaceutically appropriate base (principally water). The carbon is injected as a sterile 'ink' into the subcutaneous layer and thus is only applicable to superficial tumors [30–32]. Although the amount of ink in each injection is ~equal (30–50 μl), the dispersion of the carbon particulates in the tissue is variable when injected; the ink is made more viscous to try to minimize this dispersion; however, it can leak from some tumors, e.g., friable tumors at the surface may lose some ink during the pressure of the injection. Because the suspending ingredients subsequently dissipate leaving only the carbon particles, the particles are interspersed with tumor cells,

Table 1 Principal features of three types of EPR oximetry sensors

Types of sensor: Current status of sensor features:	Carbon particles[1] in solution (aka 'Printex and Carlo Erba ink') [28, 30–32]	LiNc-BuO crystals encapsulated in PDMS (aka 'OxyChip') [33, 34]	Multiple LiNc-BuO capsules (aka 'implantable resonator')[27, 28, 36]
Current use and max detection depth for measurements	In several clinical studies[2]; at ≤5mm in subcutaneous layer [27]	In Phase 1 safety trial in human tumors[3] [27, 35]	In preclinical studies; at any depth of tumor [27]
Physical condition and mode of implanting (invasive step)	30-50 µl pharmaceutical quality suspension that dissipates after injection; injected w 0.5 ml insulin syringe & 29½-gauge needle	Crystals encased in PDMS, formed into semi-rigid 'rod' (5.0 mm h x 0.6 mm diam); implanted w 18-gauge brachytherapy needle	Crystals encased in PDMS as ~spheroids with 0.7mm diam; 4 'spheres', separated by 7mm bio-wire coated w PDMS; implant mode TBD
Physical condition after recovering from implantation (allows non invasive measurements)	Carbon particles remain permanently in subcutaneous tissue, encapsulated in macrophage cells and macroscopic pockets	cylinder remains in tissue until scheduled surgery	Same as OxyChip
Ease of placing within tumor	Easy; applies to surface tumors so shallow; hard to control dispersion, esp if porous	May be difficult when tumor is below skin surface; location re tumor unsure until path	NA, implanted in animals during open surgery with anesthesia
Estimated volume of tissue that can be queried[4]	Variable, due to dispersion of carbon particles; surface[5] =220 $mm^{2;}$ volume=158 mm^3 (max)	Immediate vicinity of the surface of cylinder (surface ~10 mm^2)	Four 0.7mm diam 'spheres', each independently sampling at 7 mm intervals
Visibility, for resonator placement	Easily visualizable on skin; intra-cavity use may not be easily seen	Not visible; improved w ultrasound or systemic movement of resonator or with metal fiducials to improve visibility	Coupling loop on wire is visible and easily accessible

[1]Printex ink uses fine carbon black particles; Carlo Erba ink uses fine charcoal; see Flood et al. 2016 for details of ingredients.

[2]Non treatment trials to assess O_2 and response to hyperoxygenation in tumors (skin, SCC, breast) [28 patients {2 in post surg bed only} and healthy tissue (14 healthy volunteers), to assess peripheral vascular oxygen (not yet enrolling), and to predict radiation-induced fibrosis (23 patients and chemo-induced neuropathy (3 patients); [total ink sensor enrollees to date=68].

3Current IDE Phase 1 safety trial of tumors requires surgical extraction; intended use is to remain in tissue permanently [total enrollees to date: 20].

[4]Estimates are expressed as the surface area of the shape of each sensor, assuming they each query only the immediate local environment of the surface area; ink is also expressed as 3D volume since interspersed w tumor

[5]See Fig. 1. Estimates on surface and 3D are based on a cylinder 2 mm deep and 1 cm diam. Note this is a large but actual typical specimen size so represents a likely maximum volume queried.

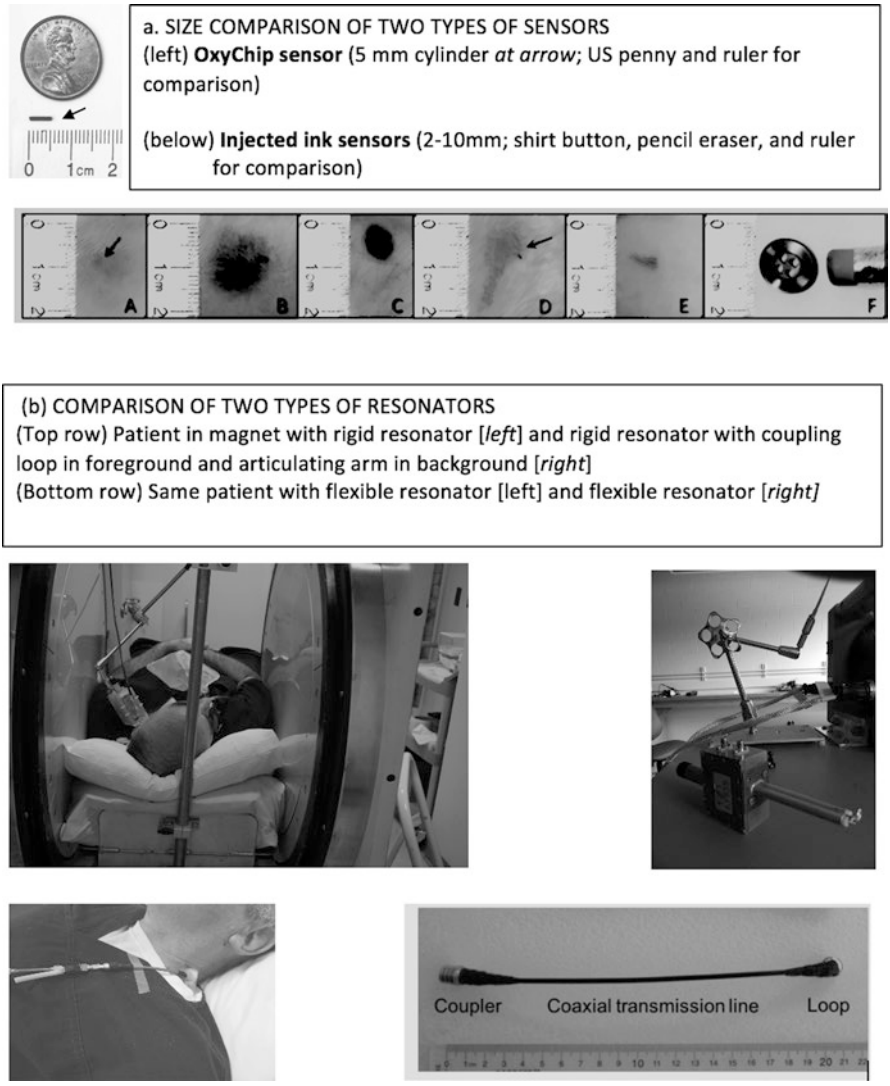


Fig. 1 Comparisons of oxygen sensors and resonators used in i*n vivo* EPR oximetry

making the tumor volume queried considerably larger than the other types of sensors (an advantage), but variably so (a disadvantage). The carbon particles are typically permanently visible (analogous to India ink used in tattooing) but can be faint because they are injected deeper than a tattoo. (Figure 1a illustrates their typical variation in size and visual appearance.) Two inks have been used to date with different carbon particulates: Printex (carbon black) and Carlo Erba (charcoal). Both are restricted to tumor depths ≤5 mm due to the sensitivity limitations of EPR oximetry for this sensor.

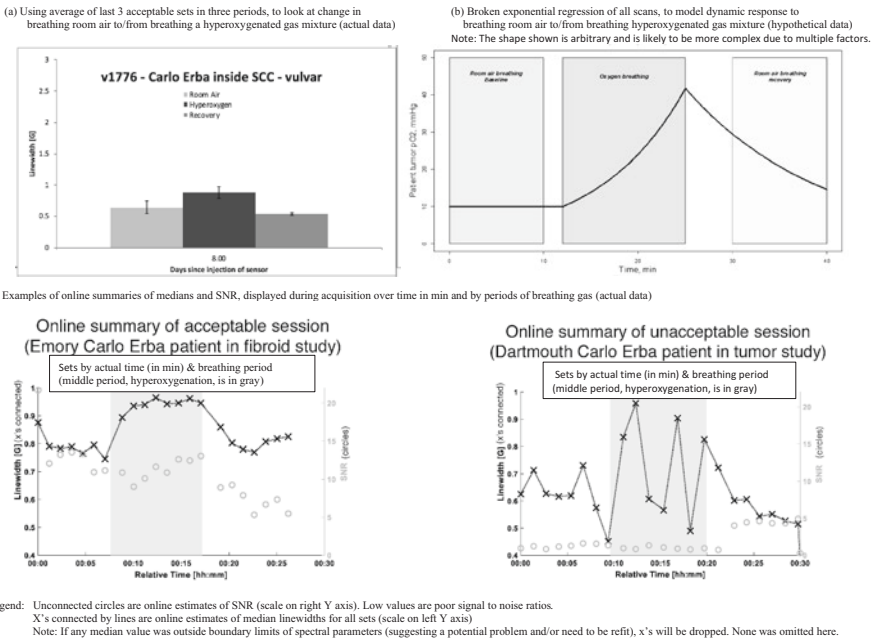
The second sensor used in humans consists of lithium octa-n-butoxynaphthalocyanine (LiNc-BuO) crystals embedded in a silicon-based organic polymer (polydimethylsiloxane or PDMS) through which O_2 can move freely [33–35]. For humans, it is molded into a solid but flexible cylinder of specific diameter and length. Since the cylinder is impenetrable by cells, it detects tissue O_2 adjacent to its surface.

Basic properties of the third sensor, the implantable resonator (see [36]), are briefly compared to the other sensors in Table 1 but not otherwise detailed here.

Once these sensors are embedded in the tissue, measurements are made with the sensing loop of a resonator placed on the surface over the sensor. Figure 1b illustrates two configurations of sensing resonators, each with a small loop. The initial clinical measurements used the rigid resonator, which was held in place for 30 min by a mechanical arm. There were several clinical disadvantages of this technique. It is difficult for the operator to place (the rigid arm is difficult to control and needs to be placed with the patient located in the magnet). It is less comfortable for the patient. It increases the likelihood of introducing measurement artifacts due to pressure from the weight on the skin (potentially interfering with the O_2 dynamics in tissues adjacent to the loop) and to being inflexible in response to patient movement (including physiological processes such as breathing). The current flexible resonator eases placement (including being placed before the patient is inside the magnet), improves comfort, and moves with breathing, thereby improving the signal to noise ratio (SNR) and minimizing technical artifacts [37].

Collecting the data: Each EPR measurement 'session' in the ongoing studies consists of ~30 min of continuous data acquisition of EPR spectra, i.e., twelve 5-sec scans are taken per min and the 12 scans are used to create 1 median, call a 'set'. The ~30 min of a standard measurement session are divided into three ~equal segments, each differing by the gas the patient is breathing, i.e., room air (~21% O_2), 100% O_2 delivered via a non-rebreather mask at a constant flowrate, followed by breathing room air again. The software then fits the median spectra so that linewidth (LW) estimates can be extracted; the software will eventually convert LW estimates to pO_2 estimates, using a calibration curve derived separately for each type of sensor. (Only LWs are displayed here.) The software flags selected parameters that are outside of the expected settings (boundaries are currently somewhat arbitrarily set) and monitors the SNR. Figure 2c is an example of the software's initial tracking of SNR and ability to display data relative to the time when the patient is breathing different levels of O_2 (after dropping flagged scans).

Figures 1 and 2c illustrate some of the real-world complexities in acquiring 30 min of continuous scans. Patients sometimes also arrive late and have medical appointments after their measurement session, thereby not leaving enough time to complete all three phases. When the mask is placed over the nose and mouth, time may elapse before measurements can be restarted. Not infrequently, the patient moves enough during this action to require a retuning step before measurements can resume. While 100% O_2 is delivered at a constant rate during the hyperoxygenation treatment period, what is actually inspired is not the same across subjects. The fraction of inspired oxygen, with the type of mask and rate of delivery used, is typically



Legend: Unconnected circles are online estimates of SNR (scale on right Y axis). Low values are poor signal to noise ratios.
X's connected by lines are online estimates of median linewidths for all sets (scale on left Y axis)
Note: If any median value was outside boundary limits of spectral parameters (suggesting a potential problem and/or need to be refit), x's will be dropped. None was omitted here.

Fig 2 Analytic models of response to hyperoxygenation for EPR oximetry measurements taken in patients

listed as 60–90%, but may be less, especially when the resonator needs to be placed on the nose or face near the mask thereby preventing a tight connection to the face. Therefore, the amount of time that a patient is breathing the hyperoxygenated gas mixture and the fraction of O_2 in the gas mixture actually inspired may vary. So, in reality, the number of scans vary from patient to patient, their quality may vary, the amount of oxygen inspired varies, and the placement within the tumor and volume queried will vary from the 'intended ideal' of data about tissue O_2.

For all these reasons, the actual data that we use to characterize whether 'a tumor is hypoxic' and whether the tissue appears to be 'responsive to hyperoxygenation' still needs to be carefully considered. How should we measure these important variables and more importantly what do our measures mean - and not mean - in regard to addressing the fundamentally important clinical questions of interest?

Five patients and one healthy volunteer (out of 68 injected to date with carbon particulates) illustrate some ways to address these questions. Specifically, we report on 2 patients in a study with ink injected into the tumor; 1 healthy subject injected in the foot; 1 breast cancer patient injected in the foot who is enrolled in a study of chemotherapy-induced peripheral neuropathy; and 2 breast cancer patients injected in the irradiated postsurgical area who are in a study of radiation-induced fibrosis.

All spectra for these patients were re-analyzed for this study to ensure that rules of selection of sessions and scans were applied consistently. Sessions were excluded for two reasons: any measurement made on the day of the injection was assumed to

be compromised by the injection/healing; any session with fewer than 6 scans per min during a breathing period was dropped as indicating an unresolved technical problem. Sets of scans were excluded if deemed 'unacceptable' by the operator of the EPR instrument at the time of the measurement.

As the primary statistic reported here, we characterize a period within a session (referred to as room air, hyperoxygenation, and recovery) by choosing the last three acceptable sets of medians and then averaging their LWs. This strategy assumes that the last few minutes of each period are a reasonable characterization of the steady-state of the actual dynamics of responding to breathing O_2-enriched gas and then responding dynamically to the return to breathing room air. Note, however, this rule may result in portraying quite different actual lengths of time since the patient started breathing O_2-enriched gas, due to delays already noted and also due to the potential occurrence of unacceptable sets at the end of the period, causing this rule to focus coincidentally on the early part of the period when the dynamic change may not be observable and therefore the response may appear to be 'insignificant'.

We have proposed other ways to look at the dynamics of responding to hyperoxygenation. One approach (a broken-exponential regression approach [38]) has the important advantage of using the full distribution of good scans across real time to examine the dynamics of the changes associated with breathing enriched oxygen and returning to room air. This model is an improvement over a continuous exponential approach for two reasons: (1) It is less impacted by practical issues that lead to real differences related to how long the patient has actually been breathing the gases. (2) It is less sensitive to whether the pO_2 has reached a steady-state plateau by the end of these dynamic periods. Figure 2b illustrates this with hypothetical data.

Another approach under consideration would have a different strength: it explicitly takes into account that a major driver of the clinical radio-sensitivity depends, not just on whether there is a significant response to breathing oxygen, but also on whether the tumor region being treated is initially hypoxic [39, 40].

Note, however, that none of these three measurement strategies fully addresses two important issues, and their consideration is important when deciding whether the assumptions underlying their applications are valid (or approximately so):

(1) [What is the 'true' pO_2?]: Tumors are well known to be heterogeneous and the distribution of pO_2 across the tumor is **not** normally distributed [41, 42]. Yet each EPR type of sensor only senses a specific subregion where it *happens* to be placed, i.e., we cannot identify a specific region within the tumor to place our sensor for maximum effect. Furthermore, if the subregion detected by the sensor happens itself to be heterogeneous, the EPR measure will still report only one value, which may or may not be weighted across time, space, and oxygen levels. The single value reported could really reflect an 'average' of the pO_2 sensed in the specific subregion, but for which there is no indication of the variation of O_2 sensed nor is the variation likely to be normally distributed (as is usually assumed when using an average). Of note for all three sensors, if there are differences in pO_2 in subregions within the volume being queried, there is a bias toward EPR's sensing the LWs from the more

hypoxic sections of the subregion. This bias may be an advantage for the clinical meaningfulness of EPR estimates because an 'average' may overreport the hypoxia.

It is therefore important to understand how much of the tumor is represented by its subregion actually being sensed. To help quantify the volume our sensors can report relative to the mass of typical tumor, we used size estimates of breast carcinomas, based on several hundred US women with invasive breast cancer [43: p2016]; size was assessed by the largest diameter of the postsurgical specimen. Michaelson et al. [43] report that the median diameter for women never-screened before diagnosis was 15 mm and for those diagnosed after a first screening was 10 mm. Applying these sizes to a cylinder shape to approximate the total tumor volume (recall that the tumor subregion sensed by each sensor in Table 1 also used a cylinder), the median total tumor volume is ~785 mm^3 (for first-screened women) or ~2650 mm^3 (for never-screened women). Comparing these estimates to the estimated subregion sensed by carbon particles (Table 1), the subregion reported by our sensor is about 5x less than the total tumor volume (for first-screened tumors) and 16x less (for never-screened tumors). However, breast cancer patients in studies using ink are not intended to be injected in tumor tissue; suitable studies of hypoxia in breast cancer would need to be conducted using our other sensors. While the superficial tumors sensible by the carbon particulates are likely to be much smaller than breast carcinomas, these comparisons of subregion-volume to total-tumor-volume illustrate the concern that the subregion sensed is only a small fraction of the actual tumor. Note, however, that the clinically important interpretation of what our measures indicate in regard to 'true hypoxia' in tumors may be less of a problem if the main clinical indicator of interest is simply to find out if the tumor appears to be responsive to hyperoxygenation or to monitor for the presence of lower levels than typical or long-term changes in levels of pO_2 in non-tumor tissues. These issues are less likely to confound preclinical studies in rodents as well, due largely to the smaller size of the animal's tumor.

(2) [Uncertainties in the data/measures of pO_2]: There are many sources for uncertainties, e.g., instrumental factors such as data acquisition, modeling of the spectra, SNR or patient factors such as movement or having a tumor not easily placed in the best location within the magnet, or operator factors such as errors in settings or differences in judgments about which sets to drop. While none of these current measures takes these uncertainties fully into account, some do a better job than others, and it will be important to improve them to better address these issues.

3 Illustrative Results

Figures 2 and 3 illustrate these statistics used in five patients and one healthy subject. Figure 2 focuses on a patient whose sensor was placed in the tumor. Figure 2a presents the primary statistic, i.e., the average of the last 3 acceptable sets and the SD. There does appear to be a response to hyperoxygenation for all but one subject, but most subjects' responsiveness varies over time.

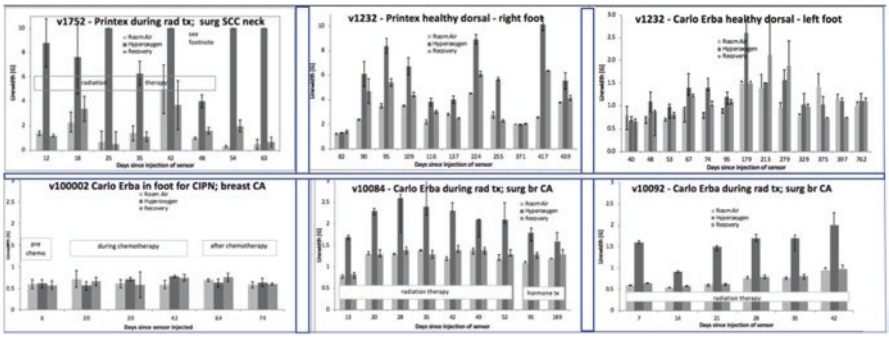


Fig. 3 Longitudinal oximetry of non-tumor tissues in healthy volunteer and cancer patients during radiation therapy or chemotherapy

Figure 2b presents an idealized model analyzing spectra over the 30 min of a measurement period, based on the broken-exponential regression technique to model the dynamics at a scan level. In preliminary analyses not presented here, this model portrays useful information about the distribution over time and its relationship to actual minutes breathing hyperoxygenated gas. Nonetheless, it identified the same sessions as showing a response to hyperoxygenation as the primary statistic.

The graphs in Fig. 2c are the online-generated portrayals of two *in vivo* measurement sessions for this patient. The individual dots portray the SNR, and the carets on the X-axis are automated 'warnings' that the spectral parameters of the scans may be out of bounds and should be investigated as to whether they warrant refitting or, in extreme cases, should be dropped from analyses. As expected, the session on the right with poorer data quality was excluded by our rules as well. While these warnings are currently based on somewhat arbitrary boundaries, they illustrate both the potential for automating the discarding or refitting on data and more importantly here, they are a reminder of the likelihood of experiencing uncertainties during measurements that, if overlooked, compromise the meaningfulness of the statistic.

Figure 3 presents data for a healthy subject and for patients for whom the sensor is deliberately *not* injected in the tumor; instead these patients are enrolled in studies of the potential for hypoxia to appear over time and during the course of therapy for their cancer, potentially predicting which patients develop debilitating side effects. Healthy subject v1232 had both types of ink injected and has been measured up to 762 days after injection, illustrating the potential for very long-term repetition of measurements. Many but far from all of v1232's sessions showed a response to hyperoxygenation and a lot of variability in the magnitude of the linewidth during the initial periods. The recovery period frequently does not return to the level of the initial period, suggesting perhaps that 10 min may not be sufficient to portray the full dynamics (at least in normal tissue in the foot). While not itself important for clinical decisions, these results suggest the dynamics of responses to breathing O_2-enriched gas bear more study if hyperoxygenation is to be used with actual therapy.

The responses of the patients in Fig. 3 are shown relative to whether they are undergoing cancer treatments. These results do not illustrate any major changes over the course of several weeks in the initial phase, nor are there obvious changes in relationship to their actively receiving treatment. Probably by coincidence, the patients receiving radiation therapy (v1752, v100084 and v100092) all showed a strong response to breathing a hyperoxygenated gas mixture while those receiving chemotherapy did not. While therapy per se may not have been related to these dynamics, the chemotherapy patient was measured in the periphery (foot), which may be slower to respond to hyperoxygenation than other regions of the body. Thus, it behooves us to remember the variability in O_2, even across types of normal tissues, and perhaps by peripheral locations. Note, however, that these remarks are not based on a systematic examination of patients, nor even all those currently enrolled.

4 Conclusions

There have been many promising developments in clinical applications of oximetry that are minimally invasive, potentially easily accomplished within usual clinical workflows and can be repeated over long periods of time. EPR sensors, particularly the carbon particulates injected as ink suspensions, have been successful in being able to assess O_2 in tissues in clinical studies and, perhaps more importantly, can assess changes in O_2 in response to hyperoxygenation. However, it is important to challenge our understandings of what our measures are actually able to tell us, in the context of what information is most needed to inform clinical decision making, whether it be to attempt to hyperoxygenate malignant tumors during radiation therapy or to monitor for indications of developing debilitating side effects. There are many important clinical studies remaining too, such as to determine whether a different method to try to increase pO_2 in the tumor would work better, at least in some patients, compared to the simple use of O_2-enriched gas mixtures. Questions such as the optimum timing of delivering hyperoxygenation or varying radiotherapy dose remain to be investigated, and, as suggested by these examples, may vary by anatomical location as well as by types of tumors or pathology being investigated [44].

This article has focused on some of the conundrums and how we are trying to improve our measures and overcome some difficulties in assessing heterogeneity in time and space with a specific sensor and EPR techniques. Despite the practical and statistical issues discussed here, it is important to keep in mind the potential advantages of EPR sensors to make repeated measurements of oxygen in the same patient from the same site. This information provides the potential to reach the long-sought goal of enhancing the effectiveness of radiation therapy by utilizing the radiation-sensitizing effects of oxygen. In order to reach this goal, it is important to continue to evaluate and improve upon both the techniques *and* the measures.

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References

1. Höckel M, Schlenger K, Aral B et al (1996) Association between tumor hypoxia and malignant progression in advanced cancer of the uterine cervix. Cancer Res 56:4509–4515
2. Gray L, Conger A, Ebert M et al (1953) The concentration of oxygen dissolved in tissues at the time of irradiation as a factor in radiotherapy. Br J Radiol 26:638–648
3. Hall E, Giaccia A (2006) Radiobiology for the radiologist, 6th edn. Lippincott William and Wilkins, Philadelphia
4. Overgaard J (2007) Hypoxic radiosensitization: adored and ignored. J Clin Oncol 25(26):4066–4074. https://doi.org/10.1200/JCO.2007.12.7878
5. Vordermark D, Horsman MR (2016) Hypoxia as a biomarker and for personalized radiation oncology. Rec Res Cancer Res 198:123–142. https://doi.org/10.1007/978-3-662-49651-0_6
6. Horsman MR, Mortensen LS, Petersen JB et al (2012) Imaging hypoxia to improve radiotherapy outcome. Nat Rev Clin Oncol 9(12):674–687. https://doi.org/10.1038/nrclinonc.2012.171
7. Tatum JL, Kelloff GJ, Gillies RJ et al (2006) Hypoxia: importance in tumor biology, noninvasive measurement by imaging, and value of its measurement in the management of cancer therapy. Int J Radiat Biol 82(10):699–757. https://doi.org/10.1080/09553000601002324
8. Turaka A, Buyyounouski MK, Hanlon AL et al (2012) Hypoxic prostate/muscle pO_2 ratio predicts for outcome in patients with localized prostate cancer: long-term results. Int J Radiat Oncol Biol Phys 82(3):e433–e439. https://doi.org/10.1016/j.ijrobp.2011.05.037
9. Nordsmark M, Bentzen SM, Rudat V et al (2005) Prognostic value of tumor oxygenation in 397 head and neck tumors after primary radiation therapy. An international multi-center study. Radiother Oncol 77(1):18–24. https://doi.org/10.1016/j.radonc.2005.06.038
10. Nordsmark M, Loncaster J, Aquino-Parsons C et al (2006) The prognostic value of pimonidazole and tumour pO_2 in human cervix carcinomas after radiation therapy: a prospective international multi-center study. Radiother Oncol 80(2):123–131. https://doi.org/10.1016/j.radonc.2006.07.010
11. Janssens GO, Rademakers SE, Terhaard CH et al (2012) Accelerated radiotherapy with carbogen and nicotinamide for laryngeal cancer: results of a phase III randomized trial. J Clin Oncol 30(15):1777–1783
12. Vaupel P, Mayer A (2017) In: Schwab M (ed) Oxygenation of tumors Encyclopedia of Cancer, 4th edn. Springer, pp 3342–3346
13. Carreau A, El Hafny-Rahbi B, Matejuk A et al (2011) Why is the partial pressure of tissues a crucial parameter? J Cell Mol Med 15(6):1239–1253
14. Vaupel P, Höckel M, Mayer A (2007) Detection and characterization of tumor hypoxia using pO_2 histography. Antioxid Redox Signal 9:1221–1235
15. Epel B, Bowman MK, Mailer C et al (2014) Absolute oxygen R1e imaging in vivo with pulse electron paramagnetic resonance. Magn Reson Red 72(2):362–368. https://doi.org/10.1002/mrm.24926160
16. Krishna MC, English S, Yamada K et al (2002) Overhauser enhanced magnetic resonance imaging for tumor oximetry: Coregistration of tumor anatomy and tissue oxygen concentration. Proc Natl Acad Sci U S A 99(4):2216–2221. https://doi.org/10.1073/pnas.042671399162

17. Magat J, Jordan BF, Cron GO et al (2010) Noninvasive mapping of spontaneous fluctuations in tumor oxygenation using 19F MRI. Med Phys 37(10):5434–5441. https://doi.org/10.1118/1.3484056
18. Bayer C, Shi K, Astner ST et al (2011) Acute versus chronic hypoxia: why a simplified classification is simply not enough. Int J. Radiat Oncol Biol Phys 80:965–968
19. Vaupel P, Mayer A (2014) Hypoxia in tumors: pathogenesis- related classification, characterization and hypoxia subtypes, and associated biological and clinical implications. Adv Exp Med Biol 812:19–24
20. Vaupel P and Multhoff G (2019) Fatal alliance of hypoxia-/HIF-1α-driven microenvironmental traits promoting cancer progression. Adv Exp Med Bio (in press)
21. Yoshida EJ, Chen H, Torres MA et al (2011) Spectrophotometer and ultrasound evaluation of late toxicity following breast-cancer radiotherapy. Med Phys 38(10):5747–5755. 3203129
22. Yoshida EJ, Chen H, Torres MA et al (2012) Reliability of quantitative ultrasonic assessment of normal-tissue toxicity in breast cancer radiotherapy. Int J Radiat Oncol Biol Phys 82(2):724–731. 3156354
23. Fukuda Y, Li Y, Segal RA (2017) A mechanistic understanding of axon degeneration in chemotherapy-induced peripheral neuropathy. Front Neurosci 11:481. https://doi.org/10.3389/fnins.2017.00481
24. Park SB, Kwok JB, Asher R et al (2017) Clinical and genetic predictors of paclitaxel neurotoxicity based on patient- versus clinician-reported incidence and severity of neurotoxicity in the ICON7 trial. Ann Oncol 28(11):2733–2740. https://doi.org/10.1093/annonc/mdx491
25. Swartz HM, Vaupel P, Williams BB et al (2019) 'Oxygen level in a tissue' – What do available measurements really report? Adv Exp Med Bio (in press)
26. Swartz HM, Clarkson RB (1998) The measurement of oxygen in vivo using EPR techniques. Phys Med Biol 43(7):1957–1975
27. Swartz HM, Williams BB, Zaki BI et al (2014) Clinical EPR: unique opportunities and some challenges. Acad Rad 21(2):197–206
28. Swartz HM, Williams BB, Hou H et al (2016) Direct and repeated clinical measurements of pO_2 for enhancing cancer therapy and other applications. Adv Exp Med Biol 923:95–104
29. Gallez B, Baudelet C, Jordan BF (2004) Assessment of tumor oxygenation by electron paramagnetic resonance: principles and applications. NMR in Biomed 17:240–262
30. Charlier N, Beghein N, Gallez B (2004) Development and evaluation of biocompatible inks for the local measurement of oxygen using in vivo EPR. NMR in Biomed 17(5):303–310
31. Desmet CM, Binh L, An T et al (2018) Characterization of a clinically used charcoal suspension for in vivo EPR oximetry. MAGMA pub online at 32:205–212. https://doi.org/10.1007/s10334-018-0704-x
32. Flood AB, Wood VA, Swartz HM (2017) Using India ink as a sensor for oximetry: evidence of its safety as a medical device. Adv Exp Med and Biol 977:297–312. https://doi.org/10.1007/978-3-319-55231-6_40
33. Pandian RP, Parinandi NL, Ilangovan G et al (2003) Novel particulate spin probe for targeted determination of oxygen in cells and tissues. Free Radic Biol Med 35:1138–1148
34. Hou H, Khan N, Gohain S et al (2018) Pre-clinical evaluation of OxyChip for long-term EPR oximetry. Biomed microdev. 1 20(2):29
35. Jarvis LA, Williams BB, Schaner PE et al (2016) Phase 1 clinical trial of OxyChip, an implantable absolute pO_2 sensor for tumor oximetry. Int J Radiat Onc Biol Phys 96(2):S109–S110
36. Caston RM, Schreiber W, Hou H et al (2017) Development of the implantable resonator system for clinical EPR oximetry. Cell Biochem-Biophy 75(3–4):275–283
37. Flood AB, Wood VA, Schreiber W et al (2017) Guidance for academics to transfer 'bench-ready' medical technology into usual clinical practice. Case study: sensors and spectrometer used in EPR oximetry. Adv Exp med bio 1072:233–239. see suppl at 314496_1_En_37_MOESM1_ESM.docx
38. Demidenko E (2019) Advanced statistics with applications in R. Hoboken: Wiley (in press)
39. Churchill-Davidson I (1964) Oxygenation in radiotherapy of malignant disease of the upper air passages. The oxygen effect of radiotherapy. Proc R Soc med 57:635–638. 4

40. Brown JM (2007) Tumor hypoxia in cancer therapy. Methods Enzymol 435:297–321. https://doi.org/10.1016/S0076-6879(07)35015-5
41. Braun RD, Lanzen JL, Snyder SA et al (2001) Comparison of tumor and normal tissue oxygen tension measurements using OxyLite or microelectrodes in rodents. Am J Physiol Heart Circ Physiol 280: H2533–H2544, 2001
42. Vaupel P, Höckel M, Mayer A (2007) Detection and characterization of tumor hypoxia using pO_2 histography. Antioxid Redox Signal 9(8):1221–1235. https://doi.org/10.1089/ars.2007.1628
43. Michaelson JS, Satija S, Kopans D et al (2003) Gauging the impact of breast carcinoma screening in terms of tumor size and death rate. Cancer 98(10):2114–2124
44. Yang Y, Lei X (2006) Towards biologically conformal radiation therapy (BCRT): selective IMRT dose escalation under the guidance of spatial biology distribution. Med Phys 32(6):1473–1484