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Measurements of Oxygen *In Vivo:* Overview and Perspectives on Methods to Measure Oxygen Within Cells and Tissues

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ABSTRACT

The goal of this review is to summarize the major techniques that currently are being applied to measure oxygenation of tissues *in vivo*. While such data would be extremely valuable experimentally and clinically, unfortunately the measurement of tissue oxygenation is nontrivial. Consequently, many different methods have been developed to try to make this measurement. This summary, largely in tabular form, covers the most pertinent aspects of the techniques and their applications, including their potential niches, limitations, and advantages. Citations are given for each method to point the reader in the direction of relevant literature. *Antioxid. Redox Signal.* 9, 1295–1301.

INTRODUCTION

While it is widely recognized that the amount of oxygen in tissues is a critically important factor in both physiology and pathophysiology, only recently that it has been possible to measure oxygen directly *in vivo*. This new capability has led to important new knowledge. It also has made less direct measurements more interpretable and more meaningful. The goal of this review is to provide a summary of the major techniques that currently are being applied to measure oxygenation of tissues *in vivo*. The summary is presented in the form of Tables 1a–c that provide short descriptions of key aspects of the various methods to make such measurements. There have been several previous overviews of the various techniques, and these remain very valuable summaries of the potential advantages and limitations of many of the techniques that are considered here (27, 36, 43, 50).

DEFINITIONS AND EXPLANATIONS OF COLUMNS IN TABLES 1a–c

Technique

The choices here were made on a practical basis, in some cases keeping together under a single heading two or more approaches that could have been listed separately, with the rationale that most of the features of the grouped methods had similar principles. Tables 1a–c attempt to list all of the major methods that provide *in vivo* data either intracellularly, interstitially, or in the microvasculature. They do not consider some less direct methods such as measurement of blood gases.

Parameters measured by each technique

There are essentially three inter-related types of measurements of oxygen in tissues:

1. direct measurement of the partial pressure of oxygen (pO_2)
2. direct measurement of the concentration of oxygen ($[O_2]$)
3. measurements that indirectly provide data on one or both of these parameters

To reach valid conclusions, it may be important to recognize which of these is being measured by each technique. It could be argued that the pO_2 relates to diffusion gradients and the $[O_2]$ to enzyme and reaction kinetics. Due to our limited knowledge about the variations in solubility across the cell and around enzymes, however, the two measurements are often interconverted using a single assumption about solubility of oxygen. The precise solubility in a given tissue is dependent on factors such as salinity, temperature, and lipid content. This

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Table 1a. Characteristics of Methods to Measure Oxygen *In Vivo*

	Technique	*Parameter measured*	*Mechanism*	*Temporal resolution*	*Site of measurement*
A	Electrodes	pO_2	Current generated by the reduction of dioxygen	1–10 s	Interstitial volume in contact with the tip.
B	Fiber-optic phosphorescent probes	pO_2	Phosphorescent lifetime dioxygen	0.5 s	Interstitial volume in contact with the tip.
C	Soluble phosphorescent probes	pO_2	Phosphorescent lifetime	Seconds	pO_2 at the site of probe and site of excitation light.
D	Near infrared monitoring of hemoglobin and myoglobin	Hb Mb	Absorption spectroscopy	20 ms–10 s intracellular myoglobin	Intravascular hemoglobin/
E	Near infrared monitoring of cytochrome oxidase	Cu_A oxidation state	Absorption spectroscopy	0.5 s	Mitochondria
F	Visible monitoring of hemoglobin and myoglobin	Hb or Mb	Absorption spectroscopy	30 ms–1 s	Intravascular (hemoglobin)/ intracellular (myoglobin)
G	Visible monitoring of mitochondrial cytochromes	$Cytaa_3$, Cytc and bc1 oxidation states	Absorption spectroscopy	1 s	Mitochondria
H	NADH and FADH fluorescence	NADH and FAD oxidation states	Fluorescence spectroscopy, 2-photon confocal fluorescence	1 s	Intracellular/ mitochondria
I	EPR oximetry based on particulates	pO_2	EPR linewidth	10 s–min	Site of particles, usually interstitial
J	EPR oximetry based on soluble materials	pO_2	EPR linewidth	Min to h (imaging)	Site of soluble molecules (usually throughout tissue)
K	NMR perfluocarbon	$[O_2]$	19F MRI	Min	Interstitial
L	NMR "BOLD" effect	Total DeoxyHb	1H MRI	200 ms possible	Intravascular
M	Proton NMR of myoglobin	Mb	1H MRS	5–15 min	Intracellular myoglobin
O	NMR Overhauser effect	$[O_2]$	Relaxation rate of protons that couple to free radicals	5–10 min	Site of soluble molecules (usually throughout tissue)

makes it difficult to come up with an accurate value. For instance, at 37°C, solubility in a tumor model was reported to be 0.93 μM/mm Hg (15), while in serum a value of 1.52 μM/mm Hg has been reported (15). A common conversion for tissue solubility is 1.35 μM/mm Hg (20), which is the solubility of oxygen in pure water at 37°C.

It is especially important to understand the basis of the more indirect measurements to know when the extrapolation to the more fundamental values is valid. But as indicated in the column title "Mechanisms", even methods that are considered to "directly" measure pO_2 or $[O_2]$ often have intrinsic assumptions which could influence the validity of the measurement. There-

TABLE 1b. CHARACTERISTICS OF METHODS TO MEASURE OXYGEN *IN VIVO*

	Spatial resolution	*Feasibility of imaging*	*Approximate resolution of pO_2*	*Potential niche and advantages*
A	100 mm–3 μm	No	0.1 mm Hg	Microelectrodes are the "gold standard for tissue oximetry. Provide distributions of PO_2 and unambiguous measurement of PO_2 under most conditions.
B	300 μm	No	0.1 mm Hg	Fast small probes which can be factory calibrated. Higher sensitivity at physiological oxygenation.
C	μL	Yes	0.1 mm Hg	Measurements can be localized to arterial, capillary, venous, or extravascular compartments.
D	mL	Yes	Undefined	Entirely noninvasive, economical, and has the potential for imaging.
E	mL	No	Undefined	Noninvasive. Measurement of oxygen at the level of mitochondria where oxygen is consumed.
F	μL	Yes	Undefined	Surface measurements are noninvasive. Economical, extremely high resolution imaging. Robust absolute quantification.
G	μL	No	Undefined	Noninvasive and highly localized. Measurement of oxygen at the leve of mitochondria where oxygen is consumed.
H	μL	Yes	Undefined	Noninvasive for surface measurements. Measurement of oxygen at the level of mitochondria where oxygen is consumed. Confocal 2-photon imaging can achieve cellular resolution in living tissue to a depth of 250 μm.
I	100 μL	No	1–5 mm Hg	Useful for repeated measurements from the same site. One probe (India ink) can be used clinically.
J	mL	Yes	7–15 mm Hg	Potential for dynamic imaging of PO_2. Repeated studies possibly feasible.
K	μL–mL	Yes	1–8 mm Hg	Potential for dynamic imaging of PO_2. Repeated studies possibly feasible.
L	μL	No	Uncertain	Rapid noninvasive imaging.
M	mL	No	1–8 mm Hg	Noninvasive measurement of deoxymyoglobin using available clinical instrumentation.
O	MRI resolution	Yes	Uncertain	Has the potential to image PO_2 in tissue using the capabilities of MRI

fore, anything that affects the parameter that is expected directly to reflect this variable would affect the validity of the data.

Mechanism for measurement

This describes the principle by which the parameters are measured. It should be noted that, in virtually all cases, the parameter really is not directly measured. Usually a physical property is measured (*e.g.*, the amount of current generated at the electrode) that under appropriate conditions is a good measure of the parameter. When the information is crucial, the validity of the assumptions should be supported by additional studies.

Temporal resolution

Assuming that technology can improve indefinitely, the intrinsic limit for time resolution for all of the techniques is milliseconds or less. Measurement times usually are longer, depending on the precision that is being sought. Also, as techniques continue to develop, the time required for measurement with a particular precision is likely to decrease

Site of measurement

The site of measurement is important to recognize because this can have a direct bearing on the interpretation of the measurement. Several of the methods involve an assumption as to the localization of the substances that are being probed. An important related consideration is the variant of the technique that is used for the actual measurement, because this may affect the region that is sampled. For example, quite different regions may be sampled depending on whether noninvasive or invasive variants of the same technique are used. In many of the methods, the detection system will have a limited volume that it probes, and the sensitivity of detection within that volume may vary, which will affect the actual sites that are being measured.

Table 1c. Characteristics of Methods to Measure Oxygen *In Vivo*

	Potential limitations	*Clinical status*	*Citations*
A	Acute tissue damage from commercial probes, probe consumes oxygen, probe calibration drifts, probe response can have slow components.	Has been used mainly in brain and tumors.	8, 53
B	Acute tissue damage. Must be used in phosphorescence lifetime mode and not intensity mode.	Systems not available for clinical use.	14, 42
C	Only probes surface tissue. Near infrared probes can measure deeper at the expense of spatial resolution.	Systems not available for clinical use.	52
D	Hb and Mb cannot be separated. Early instrumentation only mesured changes in concentration and cannot measure saturation. Newer instrumentation measures saturation. Imaging systems only measure changes in hemoglobin concentration or have poor spatial resolution and poor quantification.	Commercial instrumentation available but, even after 20 years of development, systems still mainly limited to research use.	1, 23, 39
E	Interference from hemoglobin and myoglobin can dominate the weak cytochrome oxidase signal.	Availabe in commercial NIR systems but the signal is considered unreliable.	12, 38, 45
F	Measures from the upper 1 mm of tissue, deeper tissue requires implantable probes. Cannot separate myoglobin and hemoglobin. Imaging systems only measure changes in Hb/Mb concentration, point measurements can measure absolute saturations.	Clinical spectroscopy systems are being evaluated.	4, 25, 34, 35
G	Measures from the upper 1 mm of tissue, deeper tissue requires implantable probes. Interference from Hb and Mb can dominate the weak cytochrome oxidase signal. Cannot be converted to an oxygen tension because the oxygen response is not known	Systems not available for clinical measurements.	2, 19, 24, 30, 40
H	Interference from Hb/Mb with only simplistic correction algorithms. NADH and FADH signals are both mitochondrial and cytosolic but only the mitochondrial component is directly oxygen sensitive. Signals also affected by metabolic state as well as oxygenation making the signals almost impossible to interpret	Systems not available for clinical measurements.	5, 17, 26
I	Requires administration of probe but measurements can be carried out after any trauma from the implantation has resolved. Limited depth sensitivity.	Early clinical studies using India ink in progress.	9, 28, 46–49
J	Requires administration of a probe. Resolution of low pO_2 may be difficult.	None. Agents will require clearance for use in humans.	16, 18, 33
K	Requires a perfluorocarbon injection into the site of interest. Sensitivity may be limiting.	Potential for clinical investigation.	29, 37, 44
L	Reflects total deoxyhemoglobin and therefore cannot dissociate contributions from the concentration of deoxyhemoglobin and the blood volume. the sensitivity to different vascular compartments is poorly understood. Only measures deoxyhemoglobin so results cannot be converted to an oxygen tension.	Widely used for functional brain imaging studies.	11, 41, 51
M	Separate resonances measure oxymyoglobin and deoxymyoglobin allowing an intracellular pO_2 to be calculated assuming a myoglobin P_{50}. Specific to tissues containing myoglobin.	Potentially implementable in clinical machines Limited to tissues containing myoglobin.	6, 31, 54
O	Requires administration of a paramagnetic agent. May not be sufficiently sensitive. Indirect measurement of pO_2. Requires complex instrumentation.	Requires development of new instrumentation and agents require clearance for human use.	3, 10, 32

Spatial resolution

The spatial resolution often depends on the signal/noise of the particular experimental conditions. The figures given are approximations of what might be obtainable under ideal conditions.

Feasibility of imaging

These entries describe the current state of the art, but the ability to obtain useful images varies with the experimental conditions.

Approximate resolution of oxygen (pO_2)

The pO_2 that can be resolved by the method is of great interest. Unfortunately, however, it depends greatly on a number of factors, and therefore a particular value cannot be assigned without defining the conditions of the measurement, including the time that is allocated for making the measurement. Thus, the values that are given are approximate and represent what is usually achievable by current techniques. As noted above, the values can be converted to $[O_2]$ if the solubility is known.

Potential niche and advantages

This is perhaps the most important aspect to consider when choosing a technique. In the current state of development of methods to measure oxygen in tissues, there is no technique that is clearly superior for most applications. On the other hand, the wide range of techniques that are available and the rapid improvement in many of them often enables the investigator to find a technique that is suitable for the experimental or clinical application that is being considered. For instance, using methods that rely on intrinsic (or already present in the tissue) probes tends to reduce the invasiveness but to increase some of the assumptions that are required to interpret the measurement.

Potential limitations

It is very unlikely that any technique will be free of limitations. Therefore, an understanding of the limitations of the techniques is essential for the proper selection of approaches and interpretation of the results that are obtained. The requirement for the administration of a sensing material is unlikely to be a significant limitation if there is not significant toxicity from it. The degree of invasiveness that is required may be potentially limiting in some circumstances, but not all. The most important limitations are related to the region that can be measured and potential perturbations in pO_2 caused by the method. For some applications, poor sensitivity also may be a strong limitation. Problems with interpreting the measurements in terms of the information that is sought also can be a significant limitation for the use of some techniques. This section probably is the most incomplete due to the complexities of measurement conditions and the various requirements for different applications.

Clinical status

At this time, tissue pO_2 is not measured routinely in the clinical setting, although it is used clinically in a limited number of sites. The value of making such measurements has been demonstrated, especially in oncology (21, 50). As the value and feasibility of making measurements of oxygen in tissues becomes more appreciated, it is likely that the development of clinically applicable methodology will accelerate rapidly. It is likely that the developments will proceed in two different but complementary directions. One direction is to improve the feasibility and reliability of making the measurements. This often will require increasingly complex and perhaps costly technology. The other direction is to use methods that can be widely applied, even if these methods do not provide the high quality information that the first direction will allow. The relative weighting of these two factors will vary with the clinical application. For example, precise and repeated measurements of oxygen in tumors may have important clinical implications, and therefore for this use more sophisticated methodology may be especially appropriate. Similar considerations may apply for the intraoperative measurement of oxygen in tissues. On the other hand, for clinical conditions that affect large numbers of patients whose clinical needs for such measurements is likely to persist for many years for each patient (*e.g.*, peripheral vascular disease), simpler and less precise methodology may be more appropriate.

Citations

References have been chosen to provide a start for accessing the pertinent literature and in themselves do not necessarily provide a sufficient background for understanding a particular technique. In some cases, the field is moving very fast and so a selection of recent citations was warranted. This certainly fits the field of hypoxia markers, which are injected and localized to hypoxic areas. In other more established fields, such as that of electrodes, some historical references have been included, specifically that of the Clark and Whalen electrodes. The Clark electrode included a cellophane cover (7), which minimized artifacts caused by biological materials including red cells. The Whalen electrode is a good example of a needle electrode (55). The field of BOLD (blood oxygen level dependent) MR imaging (41) is also moving quickly, fueled by the potential to map brain function with this noninvasive method. This is a very complex field. Some of the assumptions are outlined in Reference 11 and include variation in sensitivity associated with variations with field strength (13), vessel diameter (22), orientation (41), and packing of hemoglobin into red blood cells (11).

CONCLUSIONS

The appropriateness and value of a particular type of measurement of oxygen in tissues *in vivo* depends on the experimental and clinical needs for the measurement and the feasibility of applying various approaches. While it is quite likely that a particular type of measurement will be the method of choice under a particular set of circumstances, it is unlikely that any method will be intrinsically superior for most uses. The most useful data are likely to be obtained by judicious selection of at least one technique, and preferably more, that provide the type of data needed for a particular use, combined with careful interpretation of the results in terms of the type of information that is obtained.

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ABBREVIATIONS

bc1, mitochondrial complex III; Cu_A, Cu_A center of cytochrome oxidase; $Cytaa_3$, heme a and a3 centers of cytochrome oxidase; Cytc, cytochrome c; CytOx, cytochrome oxidase; EPR, electron paramagnetic resonance; Hb, hemoglobin; Mb, myoglobin; MRI, magnetic resonance imaging; MRS, magnetic resonance spectroscopy.

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