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# Forum Editorial

## On Tissue Oxygen and Hypoxia

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THE AIM OF THIS special issue is to provide an overview of methods for measuring oxygen and hypoxia in vivo and to summarize some of their most important applications. We consider the applications, both for the important information that can be obtained for the system that is described, and also to illustrate techniques for measurements of oxygen. This issue is aimed at scientists who have a need to understand the role of oxygen in the processes that they are studying. This should be a very wide audience indeed, because oxygen is so ubiquitous and involved in so many biological processes in so many ways. Paradoxically, there often has been a tendency not to consider quantitative measurements of oxygen in many processes, including oxidative damage. This tendency can lead to very significant gaps in understanding pathophysiology and physiology.

Oxygen, of course, is intrinsically involved in the generation and often the reactions of reactive oxygen species and other redox active species. Oxygen is one of the primary reactants in energy metabolism. Oxygen is increasingly being recognized as a very important variable in the evolution and treatment of many important types of pathophysiology, including tumors, wound healing, ischemia-reperfusion injury, peripheral vascular disease, and stroke. Oxygen is an especially key variable in cancer, affecting tumor progression as well as responses to therapy.

Some of the articles in this issue provide comprehensive overviews of some of the most important techniques, either from a general perspective or in the context of a particular type of pathophysiology. Other articles, while providing important insights into the measurement techniques, focus on measurements for a particular purpose. Together these papers should provide an authoritative introduction into the effective use of state-of-the-art methods to measure oxygen *in vivo*. The authors have been chosen from acknowledged leaders in the field, drawing especially from prominent members of the society that is most closely involved in measuring and understanding the roles of oxygen *in vivo*, the International Society for Oxygen Transport in Tissues (ISOTT).

Whereas oxygen remains difficult to assess *in vivo* both clinically and experimentally, with the increased awareness of the value of such measurements there has been a very significant increase in the development of approaches to assess oxygen levels *in vivo* under both steady state and dynamic conditions. In an *in vivo* study, it is unlikely that a single type of measurement will be clearly the most accurate under most circumstances, and therefore a single gold standard is unlikely. It should be recognized, however, that it is difficult to compare the results obtained with different techniques. The various methods may differ in their sensitivity, accuracy, and ability to measure repetitively. Clearly one needs to consider carefully the strengths and weaknesses of each type of measurement, and try to gain insights into the uncertainties. But differences in sensitivity and accuracy are not the largest challenge. For measurements of oxygen *in vivo*, the most problematic aspect is that the various types of measurements usually do not measure the same thing. This is because various techniques usually differ significantly in one or more of the following aspects:

1. The parameter that they measure
2. The spatial dimension that they probe directly
3. The time resolution of the measurements
4. The compartment in which the measurement is made
5. The perturbations that occur as a consequence of the measurement

For each technique, there is a tradeoff between resolution and the quality of the data that are available. In a very simplistic way, one can note that if the same amount of information is available, having it in one or a few voxels will provide better accuracy, while having it in many voxels will provide additional information on the distribution of oxygen, but at a cost of the signal/noise in each voxel. The amount of resolution that is appropriate depends very much on the specific goals of the studies, and more resolution is not always better. Sometimes the most useful data will be obtained by measurement at one or a few points with high precision and high resolution of time. It also should be kept in mind that it is very unlikely that any technique or even combination of techniques will completely resolve the heterogeneity of oxygen *in vivo*, because the

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variation is significant over spatial dimensions of less than the diameter of a cell, and there also is variation over both very short times and over longer time intervals as well (1).

The characteristics of the various techniques are described in detail in the article by Springett and Swartz (10). This relatively short article provides a very useful overview for understanding the nature of the various types of measurements, including their advantages, disadvantages, and potential niche.

Some of the most versatile and potentially important techniques for making measurements of oxygen *in vivo* are discussed in detail in several of the articles. The article by Vaupel *et al*. (12) provides a comprehensive review of the result of the method (oxygen electrodes), which has sometimes been termed the "gold standard" for measurements of oxygen *in vivo* and which has been the most applied method in human subjects. The authors provide an excellent illustration of the value of measurements of oxygen *in vivo*. They also provide very important insights as to the complexities of such measurements and, especially, how one needs to view the results in context. Summarizing data from several different institutions, they show both that the overall trends are very consistent and therefore of great value for clinical purposes, but also that the absolute values that are obtained vary among institutions, even though the institutions built up considerable expertise and experience in the technique. The results obtained with the oxygen electrodes have been a powerful incentive for the development of techniques that can provide measurements of oxygen *in vivo* less invasively and with less technical challenges for making the measurements consistently.

In his article (2), Jeff Dunn provides an overview of the many nuclear magnetic resonance (NMR) techniques that can be brought to bear on measurements of oxygen. Using a systems perspective, he illustrates how different NMR techniques can be utilized to follow oxygen as it moves from outside the body to the cell. A great advantage of NMR techniques is their wide availability and their usually noninvasive nature (although one of the most specific and sensitive techniques, using perfluorocarbon to measure oxygen in tumors, does require injection of the material into the site of interest). A second advantage is the wide range of approaches available using NMR techniques and, increasingly, combining them with other techniques. The latter helps to overcome the major disadvantage of the existing NMR techniques: these generally do not measure oxygen directly but instead measure parameters that are related to oxygen. The relationship is seldom direct. For example, the widely used blood oxygen level dependent (BOLD) technique measures the total amount of deoxyhemoglobin in the volume that is studied, so changes in the BOLD signal can occur through both changes in the saturation of hemoglobin and in blood volume and further, there is no simple relationship between oxygen availability in the circulatory system and oxygen in the cells of interest.

Optical techniques are considered in the context of measuring oxygen in the breast in the article by Srinivasan *et al*. (11). They review the capabilities of a new stand-alone near-infrared (NIR) optical tomography system applied to measurements of oxygen in the breast. This article also emphasizes an important direction for *in vivo* measurements that is leading to significant improvements for such measurements: the use of multiple modalities to obtain the information. In this case they combine the ability of NIR to provide localized data on the physiological parameter of interest (oxygen saturation) with the ability of NMR to resolve anatomy.

The use of electron paramagnetic resonance (EPR or ESR) is an important new technique that appears to provide some unique advantages for several types of measurements of oxygen, including measurements in human subjects. Both imaging and spectroscopic techniques have been developed. The article by Matsumoto *et al*. (7) provides a comprehensive overview of the EPR-related imaging techniques, including hybrid techniques that combine EPR with NMR. These techniques are providing some very useful information *in vivo* in animals (see, for example, the article by Kutala *et al*. (6) in this issue) and have some potential for use in human subjects some time in the future.

A distinct and complementary variant of EPR oximetry, based on the use of particulate paramagnetic materials is described by Khan *et al*. (5). This approach does not provide full spatial information, although it can obtain data from several sites simultaneously. Its niche is that it can make *repeated* measurements from the same site for periods of up to at least several years. This technique already has been introduced into clinical medicine, and NIH supported trials are underway for measurements in patients with tumors and with peripheral vascular disease.

The article by Hopf and Rollins (3) provides excellent insights into the value of measurements of oxygen to understanding fundamental processes such as wound healing. As they note, understanding the role of oxygen is one of the keys to understanding the healing of wounds and then to improve the healing process. This need for information generates the need for appropriate techniques and, as they conclude, "Our understanding of the role of oxygen in wound healing has been fueled by tissue oximetry. Advances in technology will lead to further advances in the management of patients with wounds." This theme is reinforced and extended in the provocative article by Hunt *et al*. (4). They report that lactate can act as an important signal for the angiogenesis that is needed for robust wound healing, and most interestingly, this effect occurs even in the presence of oxygen. This research provides both a general conceptual framework for understanding the effects of oxygen at the molecule level as well as very specific aspects related to wound healing. It highlights the complexity of cell signaling and reminds us again that careful studies are needed to understand the mechanisms thoroughly; in this case, they had the insights to look for specific functions of lactate rather than accept the conventional interpretation that it just reflects metabolism during hypoxia.

The conclusions on the importance of measuring oxygen and then using this knowledge to enhance therapy also are reached in the article by Kutala *et al.* (6). They show that reperfusion injury and postischemic cardiac function are related to the quantity and delivery of oxygen during reperfusion. They provide substantial evidence suggesting that controlled reoxygenation may ameliorate postischemic cardiac dysfunction.

Moon *et al*. (8) consider in detail a molecular biological approach to measuring oxygen *in vivo*. They provide an extensive review of the results of using hypoxia-sensitive genes to indicate the presence of hypoxia and conclude that, despite the promise of such an approach, so far it has not been possible to achieve quantitative results with this approach. I believe this is

a very important paper because, in addition to the excellent content on the subject, it also provides an important generalizable indication that the use of activation of genes to follow complex physiological processes, such as the occurrence of hypoxia, may be inherently very difficult. This is because of the complex and interrelated pathways for gene activation that are necessary for appropriate control of cells; therefore, it is unlikely that a pattern of gene activation will be unique for a particular alteration in the environment of the cell, and it will be especially unlikely that there will be a direct quantitative relationship with a particular variable such as hypoxia.

When considering oxygen levels *in vivo,* whether in terms of physiology or pathophysiology or therapeutics, it is important to keep in mind that oxygen levels *always are heterogeneous in living systems* (1). This is described very well by Ndubuizu and LaManna (9), who point out that although certain large-scale methods can provide reproducible average brain $pO_2$ measurements, there can be no useful concept of a characteristic oxygen tension or meaningful average value for tissue oxygen in normal brain on a microregional level. Similar insights for tumors are provided by the articles by Moon *et al*. (8) and Vaupel *et al*. (12). The article by Ndubuizu and LaManna (9) also provides excellent insights into how the various types of measurements of oxygen can be utilized to provide a meaningful picture of the distributions and inter-related changes in oxygen within tissues.

## CONCLUSIONS

The ability to measure oxygen *in vivo* has increased as the value of having such measurements has become more widely appreciated. There certainly is no "gold standard" that applies to all needs, and it is important that one understands both the nature of the problem as well as the nature of the measuring technique to obtain the most useful information. In many cases, the best approach will be to use a combination of methods. With good measures of oxygen and closely related parameters, understanding of complex and important medical problems such as cancer, wound healing, and ischemia–reperfusion injury can be advanced significantly as can understanding of complex physiological aspects of the functioning of critical organs, such as the heart and brain. While the techniques are most advanced for studies in experimental animals, there has been great progress recently in moving these techniques into clinical medicine.

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