Clinical EPR:

Unique Opportunities and Some Challenges

Harold M. Swartz, MD, PhD, MSPH, Benjamin B. Williams, PhD, Bassem I. Zaki, MD, Alan C. Hartford, MD, PhD, Lesley A. Jarvis, MD, PhD, Eunice Y. Chen, MD, PhD, Richard J. Comi, MD, Marc S. Ernstoff, MD, Huagang Hou, MD, Nadeem Khan, PhD, Steven G. Swarts, PhD, Ann B. Flood, PhD, Periannan Kuppusamy, PhD

Electron paramagnetic resonance (EPR) spectroscopy has been well established as a viable technique for measurement of free radicals and oxygen in biological systems, from in vitro cellular systems to in vivo small animal models of disease. However, the use of EPR in human subjects in the clinical setting, although attractive for a variety of important applications such as oxygen measurement, is challenged with several factors including the need for instrumentation customized for human subjects, probe, and regulatory constraints. This article describes the rationale and development of the first clinical EPR systems for two important clinical applications, namely, measurement of tissue oxygen (oximetry) and radiation dose (dosimetry) in humans. The clinical spectrometers operate at 1.2 GHz frequency and use surface-loop resonators capable of providing topical measurements up to 1 cm depth in tissues. Tissue pO_2 measurements can be carried out noninvasively and repeatedly after placement of an oxygen-sensitive paramagnetic material (currently India ink) at the site of interest. Our EPR dosimetry system is capable of measuring radiation-induced free radicals in the tooth of irradiated human subjects to determine the exposure dose. These developments offer potential opportunities for clinical dosimetry and oximetry, which include guiding therapy for individual patients with tumors or vascular disease by monitoring of tissue oxygenation. Further work is in progress to translate this unique technology to routine clinical practice.

Key Words: Electron paramagnetic resonance; free radical; oxygen; hyperoxygenation; tumor therapy; radiation therapy; dosimetry.

Electron paramagnetic resonance (EPR) (also called electron spin resonance) spectroscopy shares many of the features of nuclear magnetic resonance (NMR), spectroscopy and magnetic resonance imaging (MRI) including underlying principles, discovery period, and their evolution to become an indispensable tool for in vivo biological applications. Particularly, proton MRI has rapidly emerged to become a unique device for noninvasive measurement (imaging) of tissue pathophysiology in the clinic. On the other hand, EPR, which relies on paramagnetic species with unpaired electrons, despite its superiority to NMR in terms of detection sensitivity, has not advanced to use for pertinent clinical applications for a variety of reasons. The most important impediments are the lack of adequate levels of paramagnetic species in biological systems, shorter relaxation times of unpaired electrons when compared to protons, and the need to use microwave radiation as source of excitation. In spite of the limitations, the last three decades have seen some innovative and concerted successful progress that has developed this unique technology for making very useful measurements in living systems (1–3). The most pertinent advances include the development of low-frequency instrumentation including lumped-circuit resonators, time-domain detection, imaging capabilities, and molecular probes for extracting specific information of interest from tissues of both animal models and human (4). The technological advances have now reached a stage where useful clinical measurements such as tissue oxygenation (oximetry) or radiation exposure (dosimetry) in human subjects have become a reality (Table 1). This article presents an overview of the unique opportunities of the EPR technology for measurements in humans and challenges that need to be addressed before it can achieve widespread acceptance as a useful clinical device. Two potential areas of clinical application, namely, oximetry and dosimetry, for which substantial progresses have been made are highlighted.

MEASUREMENT OF TISSUE OXYGENATION (OXIMETRY)

Although the discovery of oxygen was made in the 18th century, measurements of oxygen concentration (oximetry) in living systems (in vivo) are only recent phenomena. Some measurements were made in the 1960s, but it was in the late 1980s when the computerized polarographic needle electrode

Acad Radiol 2014; 21:197–206

From the Department of Radiology, Geisel School of Medicine at Dartmouth, Dartmouth College, 48 Lafayette Street, Lebanon, NH 03766 (H.M.S., B.B.W., H.H., N.K., A.B.F., P.K.); Departments of Medicine (B.I.Z., A.C.H., L.A.J., R.J.C., M.S.E.) and Surgery (E.Y.C.), Geisel School of Medicine at Dartmouth, Dartmouth College, Hanover, NH; and Dept. of Radiation Oncology, University of Florida, Gainesville, FL (S.G.S.). Received August 15, 2013; accepted October 14, 2013. **Address correspondence to:** H.M.S. e-mail: Harold.M.Swartz@Dartmouth.edu

http://dx.doi.org/10.1016/j.acra.2013.10.011

TABLE 1. Clinical EPR at Dartmouth

Clinical Problem	Parameter to Be Measured	Status of Measurements in Human Subjects	Rationale for Using In Vivo EPR Measurements
Dosimetry	Radiation-induced free radicals	Extensive measurements underway	Fills unique niche for emergency dosimetry based on physical parameter
Peripheral vascular disease	Oxygen at sites of likely pathologies	Measurements underway in normal volunteers; patients to be studied in near future	The pO_2 in the tissues is the most significant pathophysiological variable; no other method available to make such direct measurements. The pO_2 measurements will facilitate evaluating progression of disease and success of therapeutic intervention
Cancer	pO_2 in tumors	Measurements underway in patients with superficial tumors; IDEs submitted for permission to start studies with embedded materials	The response of tumors to cytotoxic therapy, especially ionizing radiation, is critically dependent on pO_2. Antitumor therapies are given repeatedly and often change pO_2. Knowledge of the changes in individual patients would significantly optimize the timing of the therapy
Wound healing	pO_2 at various sites in wounds, perhaps reactive oxygen species	Existing clinical protocols use an apparatus compatible with EPR measurements.	The pO_2 is a critical variable for successful healing of wounds. Direct measurements would identify patients likely to have poor healing and follow responses to therapy
Radiation-induced fibrosis	pO_2 in irradiated tumor beds and peripheral normal tissue	Initial studies have been started	Radiation-induced hypoxia may play a critical role in the signaling of proinflammatory, profibrotic, and proangiogenic growth factors and cytokines that lead to tissue fibrosis.

EPR, electron paramagnetic resonance; IDE, investigational device exemption.

system became available that it was used extensively to assess the oxygenation in tumors clinically. The use of this technique helped to establish the role of hypoxia in the efficacy of radiation therapy (5). Now, there are several potentially clinically useful methods (6) that are based on other principles, including fluorescence quenching, phosphorescence, optical detection, immunohistochemical, and magnetic resonance techniques. Some of the important criteria for improvements in the ability to make successful clinical measurements of oxygenation include minimal or no invasiveness, capability to make repeated measurements, accessibility to the region of interest, appropriate spatial resolution, adequate depth of measurement, accuracy and robustness of measurements, usefulness of the parameter reported for clinical purposes, measurement time consistent with use in patients, ease of use in the clinical setting, and potential for the instrumentation to be commercially available. It is especially important that the method should enable repeated measurements from the region of interest to follow the changes in oxygenation over a period of time, preferably for up to several weeks, months, or even years (7,8). The technique should also provide appropriate spatial and temporal resolution. The depth of measurement (penetration) and accessibility to the region of interest are some of the important factors for establishing the scope of applicability of the technique.

EXISTING METHODS FOR TISSUE OXIMETRY IN VIVO

Although a few techniques are available that can provide direct measurements of tissue pO_2 (especially oxygen polarographic electrodes, the OxyLite fluorescence-quenching technique, and direct injection of oxygen-sensitive NMR probes based on fluorine (9–11)), these techniques have the disadvantage