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Clinical EPR: Unique Opportunities and Some Challenges

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Abstract

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 paper 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 pO₂ 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.

Keywords

Electron Paramagnetic Resonance; free radical; oxygen; hyperoxygenation; tumor therapy; radiation therapy; dosimetry

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Introduction

Electron paramagnetic resonance (EPR) (also called electron spin resonance, (ESR)) 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. 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 a recent phenomenon. Some measurements were made in the 1960s, but it was in the late 1980s when the computerized polarographic needle electrode 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 in order 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

While 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 of not being able to be used repeatedly. The inability to make repeated measurements is a disadvantage because the most valuable clinical usefulness depends on obtaining repeated measurements so that changes due to the treatment and the disease process can be followed. There are also some concerns about the potential for artifacts because the invasiveness of these techniques can cause perturbations precisely at the time that the measurements are being made [12–16]. To date, only the polarographic method has been systematically used in humans [17].

Some widely available non-invasive techniques to assess oxygenation provide data on parameters that, while related to tissue pO_2 , do not measure pO_2 directly. This especially includes techniques such as blood oxygen level dependent (BOLD) MRI, NMR proton spectroscopy, diffusion-weighted imaging (DWI) MRI, duplex Doppler ultrasound, ^{18}F -Miso positron emission tomography (PET), and near-infrared (NIR) measurements of hemoglobin. These techniques can provide information on the saturation of hemoglobin within the vascular system or hemodynamics or redox-related metabolites or reactions, but, while this can be valuable to know, it does not provide direct quantification of the pO_2 in the site of interest and surrounding tissues. The clinical importance of direct measurement of pO_2 is especially high in tumors, where typically the microvasculature is complex and irregular and therefore is very difficult to extrapolate these indirect measurements to the pO_2 in the tumor [18, 19]. EPR oximetry could provide the data to facilitate relating these indirect methods to the actual tumor or tissue pO_2 , thereby clarifying the conditions under which these other methods can provide the desired clinically relevant information on pO_2 .

EPR Oximetry for biological systems including viable tissues

EPR oximetry refers to the measurement of oxygen concentration by EPR spectroscopy [20]. 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. In most cases the relaxation rate of the spin-probe increases proportionately as a function of oxygen content (concentration or pressure) [20, 21], but other mechanisms also can occur. The increased spin-spin relaxation rate results in increased line-broadening. The fact that the linewidths of EPR resonance lines correlate with oxygen concentration has been used in a variety of biological settings [22–35]. The development of low-frequency EPR instrumentation at L-band (1–2 GHz) and even lower frequencies (600 MHz and 300 MHz) has made it possible to perform EPR oximetry measurements on complex biological systems such as intact animals and isolated functioning organs [23, 36, 37]. Unfortunately, clinical EPR must confront the fact that the depth of penetration will continue to be a limiting factor, unless further advances in hardware design and their applications are successful. Generally speaking, noninvasive EPR measurements are limited to a penetration depth on the order of 1 cm at L-band (~1.2 GHz). Penetration depth can be increased to 8 cm or more by lowering the frequency to the 300–600 MHz range. The tradeoff, however, is that the signal-to-noise ratio significantly decreases at these lower frequencies, causing a decrease in spatial resolution and accuracy of pO_2 measurements. Implantable resonators are one potential way to overcome this restriction for the successful clinical application of EPR for oximetry measurements at deeper sites [38].

Paramagnetic probes for possible use in clinical EPR oximetry

Measurement of oxygen-induced EPR line-broadening requires the presence of a suitable paramagnetic probe in the tissue region of interest. Two types of probes are used: (i) soluble probes that report the concentration of dissolved oxygen and (ii) particulate probes that measure partial pressure of oxygen (pO_2) in the milieu. Considerable progress has been made in the development and use of both types of probes. The soluble probes provide the potential for imaging, but have the potential limitations of being limited in their sensitivity and the ability to repeat the measurements. Nevertheless they have been very successful in a number of applications in preclinical models. Because they need to be administered directly their translation to clinical applications hinges on the success of having a sponsor who will be willing and able to invest the many hundreds of millions of dollars required for achieving regulatory approval for their use in human subjects.

There are certain advantages in using particulate probes including that they can more readily be approved for use in human subjects as described below. The other attractive features of particulate probes include (i) high resolution - resolutions in the range of 0.1 torr (mmHg) can be obtained; (ii) suitability for repeated measurements in vivo without reintroduction of the probe into the tissue; (iii) non-invasive measurement - may require one-time introduction of the probe; however, subsequent measurements are performed under noninvasive conditions (iv) accuracy - highly reproducible and correlate closely with measurements by other methods; (v) localized measurements - the measurement is made from a single voxel/region containing the particulate - thus the spatial resolution is the size of the particulate deposit; (vi) insolubility in aqueous solvents; (vii) no effect of the various biological oxidoreductants, pH, temperature etc.; (viii) Nontoxic - the probes are very inert in biological systems; (ix) temporal response is very good, usually less than a seconds (x) respond to partial pressure of oxygen (pO_2), rather than concentration of oxygen, the latter may be quite heterogeneous in cellular/tissue environments because of the solubility of oxygen and hence calibration can be difficult. The use of both the naturally occurring (coals, fusinite), semisynthetic (India ink, sugar chars, carbon blacks) and purely synthetic crystalline materials, e.g., lithium phthalocyanine (LiPc), lithium naphthalocyanine (LiNc), and lithium octabutoxynaphthalocyanine (LiNc-BuO) have been reported for EPR oximetry [33, 39–47].

The particulate probes typically are introduced into tissues using a small gauge needle (21 – 25 gauge), often several days prior to measurement in order to minimize trauma and allow for healing and incorporation of the material into the tissue prior to pO_2 measurement. An advantage of the particulate approach is that once the probes have been introduced, they may be used to make repeated measurements at the same location without the need to introduce additional material or wait for introduced material to be cleared. This characteristic is particularly useful for the monitoring of tissue oxygenation during the development of disease or in response to therapy, as needed for the optimal treatment of solid tumor cancers and peripheral vascular disease.

India ink has been the first probe used for oxygen measurements in human [40]. India ink has a long history of clinical use as an anatomic marker for surgery and radiotherapy and, fortuitously, the suspended carbon black particles contain stable radical species at sufficient concentrations with EPR spectra that are highly sensitive to the presence of oxygen [43, 48]. Charcoal, a naturally occurring carbonaceous material, has been extensively characterized for EPR oximetry in animal models [49–51]. Charcoal has been approved for clinical use (as a tissue marker) in Europe. Despite their availability for clinical use, the carbon-based oxygen sensors have some limitations insensitivity to oxygen and if used as suspensions may diffuse. Alternatively, purely synthetic materials such as lithium phthalocyanine [33],

or naphthalocyanine [52] and derivatives [45, 47], are ideally-suited for clinical EPR oximetry. However, these materials need regulatory approvals for use in human. The most notable drawback and potential barrier to the use of these materials in clinical EPR applications is the need to leave these particulate probe materials permanently in the tissue, which may present practical barriers for obtaining approval for use in human subjects. Therefore, we are developing an alternative approach for their clinical use: to embed them in biocompatible materials that have high oxygen permeability [49, 53–55]. The probes are effectively shielded from interaction with the biological *milieu* that could result in biochemical degradation and breakdown, as well as local and/or systemic toxicity effects that may occur due to these reactions. The implants could be left in the tissue or removed when no longer needed.

Although India ink has been the only sensor currently in use for measurement in humans, the crystalline materials such as LiPc and LiNc-BuO have some potential advantages. The crystalline sensors have significantly higher EPR detection sensitivity, largely due to higher paramagnetic spin content [33, 43, 47] and narrower lineshape when compared to India ink. The narrower lineshape of the crystalline sensors also contribute to their higher oxygen resolutions, typically in the range of 0.2–0.5 mmHg. Our laboratory is keenly focused on developing the crystalline sensors for use in clinical oximetry.

Multisite oximetry

Traditional EPR oximetry provides average pO_2 estimates based on the summed signals detected from spins within the sensitive volume of the resonator. In order to provide additional spatial resolution of pO_2 within the tissues, we have developed procedures for simultaneous measurements from multiple implants, as well as implantable resonators with multiple sensing tips that are spatially distributed within the tissue [27, 38, 56]. The multi-site measurement technique uses magnetic field gradients to spectrally distinguish their respective signals. A number of analytic methods have been developed [57–59] which exploit this sparseness in spatial spin distribution to limit the number of degrees of freedom involved in pO_2 estimation and significantly reduce the data acquisition time as compared to conventional spectral-spatial imaging. Our laboratory has pioneered an approach coined “multi-site oximetry” where sparseness of the spin distribution is exploited [57, 58].

Clinical potential of EPR oximetry

Although several existing other methods are utilized to try to measure oxygen concentrations in vivo in human subjects, a suitable technique for noninvasive and repeated direct measurements of oxygen in the same tissue or cells on a temporal scale is not available currently. While electrode techniques have evolved as the standard methods for measurement of oxygen, they are complex to use and can generate analytical artifacts during assay procedures at the freshly probed sites and they are not suitable for repeated measurements [17]. Near-infrared and magnetic resonance techniques such as nuclear magnetic resonance, blood oxygen level-dependent (BOLD) magnetic resonance imaging, Overhauser-enhanced magnetic resonance imaging (OMRI) are noninvasive methods. However, they do not report the absolute values of oxygen concentration in the tissues [10, 60–64]. EPR oximetry enables reliable and accurate measurements of the pO_2 [20]. EPR oximetry can measure directly in the tissue and can be done at the actual site(s) of interest. The placement of the paramagnetic material directly at the site of interest, where it remains and can be interrogated after any temporary perturbation has resolved, also avoids potential concerns about damaging the tissue at the time of measurement [8]. The ability of EPR oximetry to make repeated measurements from localized sites provides a very important capability that can enable critical aspects of a number of biomedical applications.

Constraints for Clinical EPR development

At present the EPR systems for use in laboratory animals have been fairly well developed using low-frequency (1.2 GHz or below) source and lumped circuit resonators capable of providing whole-body measurements including imaging [65]. However, clinical EPR is faced with several constraints and challenges with respect to instrumentation, probe administration, and regulatory issues [2, 3, 8]. Magnets with large gap and sufficient magnetic-field homogeneity are needed to accommodate and enable measurements in human subject. Our laboratory has succeeded in using large permanent magnets (gap of 50 cm) with field homogeneity suitable for even the very narrow linewidth of anoxic lithium phthalocyanine (20 milligauss) for whole-body oximetry measurements (Figure 1). We have also developed magnet designs customized for head, and for dosimetry measurements in human tooth in vivo. The applicability of these clinical EPR systems have been demonstrated, to date, in a large number of normal volunteers and more than 30 patients. Another major constraint for clinical EPR oximetry is the need to use an exogenous probe, which is placed in the desired location of the tissue, using a minimally invasive surgical procedure. The probe, once implanted, will stay in the tissue for a desired length of period or permanently enabling repeated measurements of pO_2 . This raises safety and ethical concerns for use in human and thus necessitates elaborative studies, which are both expensive and time-consuming before getting FDA clearance. Of the oximetry probes characterized in animal models, India ink has previously been approved for clinical use, and hence our laboratory has started to use India ink for clinical measurements. Although we have alternative probes, based on lithium phthalocyanine (LiPc) derivatives [33, 47, 66] that are much superior to India ink in terms on oxygen sensitivity, and other desirable properties their use in human awaits regulatory approval. One important consideration towards increasing the safety of the implanted probe material, which is usually in the form of microcrystalline powder, is to encapsulate it in a biocompatible, oxygen-permeable polymer [54, 55, 67–69]. The encapsulation will not only hold the probe particulates from smearing or migration over a period of time but also avoid direct contact of the probe with the cells. We have used polydimethylsiloxane (PDMS), a silicone polymer with properties desirable for encapsulation of oximetry probes. It is a biocompatible, highly flexible, highly oxygen permeable [70] and has been used in a wide range of medical device and health care applications [71]. PDMS has been approved for use in human subjects and is one of the reference materials provided by the National Heart Lung and Blood Institute for standardized biocompatibility testing [72]. We used PDMS to encapsulate LiNc-BuO and developed the implants in the form of thin films (OxyChip). The efficacy and safety of OxyChip have been well characterized in animal models [55, 69]. We have taken steps to obtain investigational device exemption status from FDA for testing the OxyChips in human subjects. The OxyChip implants will be used for subcutaneous and tissue measurements up to a depth of 10 mm. For measurements deeper than 10 mm we will use implantable resonators, which we have developed and demonstrated in animal models [27, 38, 56]. The implantable resonators (Figure 2) are capable of deep tissue pO_2 measurements anywhere in the body, and at multiple sites, simultaneously. FDA clearance for the use of implantable resonator in human is expected be less problematic.

Other than instrumentation and probe, there are also additional challenges in the use of clinical EPR. Translation of the new technology for clinical practice involves a variety of factors, most importantly, the human factors including operators (technicians), physicians, and patients and the need to fit the measurements into the established clinical routines or emergency response. The EPR Center at Dartmouth is also working on the translational aspects of this technology for clinical acceptance and practice.

Clinical oximetry measurements

We have demonstrated that oxygen measurements can be made in human subjects in superficial tumors using India ink, the only currently approved material for use in human subjects. Extensive pre-clinical and clinical development has shown the feasibility and safety of repeated oxygen measurements for easily-accessible, subcutaneous sites. The development of India ink as a clinically-applicable oxygen reporter was carried out jointly by the Dartmouth laboratory and Dr. Gallez' research group in Belgium [3, 40, 41, 43, 48, 73–75]. At the Dartmouth EPR Center, EPR oximetry measurements in human subjects are performed using the clinical whole-body L-band EPR spectrometer [3, 8, 48, 76]. This spectrometer consists of a 420-G permanent magnet, magnetic field modulation and sweep coils, RF detection system including surface-loop resonators, and computer control [1, 8]. The spectrometer operates at an L-band frequency (~1.2 GHz), where a practical compromise between RF penetration depth and EPR sensitivity is achieved. At this frequency RF penetration depths of approximately 1 cm are achieved. Data on oxygen measurements in 11 superficial tumors at different sites in 10 patients is summarized in Table 2

The experimental procedure was relatively simple in order to make the measurements under conditions that could readily be incorporated into normal clinical processes. The India ink was inserted into the tumors in the outpatient clinical, with local anesthesia and sterile procedures. A 21 to 23-gauge needle was used to insert 10 – 25 microliters of a sterilized slurry of India Ink, whose response to oxygen had been well calibrated *in vitro* and *in vivo* in animals. One - five days later a baseline measurement was made and then the patient was given an enriched oxygen mixture to breathe through a regular simple face mask. EPR measurements were made continuously while breathing the enriched oxygen mixture and then when the patient returned to breathing room air. The procedure took less than an hour in total and was very well tolerated by all subjects. The data as summarized in Table 2 lead to several tentative conclusions: (i) The limited amount of data and small number of tumor types permit only qualitative implications to be drawn; (ii) The tumors varied considerably in their baseline pO_2 , which ranged from 0 to 10 torr; (iii) The subjects varied considerably as to whether their tumors responded to increased oxygen in the breathing gas (four out of 11 did not respond); (iv) The amount of the increase in tumor oxygen varied widely among the responders (3 to 100 torr).

Even with such a limited amount of data, these results indicate that the ability to make repeated measurements of oxygen in tumors could be quite useful clinically. The measurements could be made under conditions compatible with usual clinical practice. The observation that a number of tumors did not respond at all to increases in the amount of oxygen in the breathing gas illustrates how misleading it could be to try to evaluate the ability of hyperoxygenation strategies to improve outcomes without being able to detect whether the treatment did actually change pO_2 in the tumor. The potential for individualizing therapy also is illustrated because the time patterns of increased oxygen and return to normal varied among the subjects (data not shown).

The other systematic clinical oximetry study that has been initiated is directed towards the use of EPR oximetry to follow oxygen levels in critical tissues in patients with peripheral vascular disease. Our initial studies have been limited to normal subjects with injections into the subcutaneous tissues. We have been able to make repeated measurements of tissue oxygen in the foot at the sites where the major pathophysiology usually occurs, under the first metatarsal [3]. Measurements have been made successfully for more than five years from an initial single injection, including continuing responses to temporary interruption of the blood flow and to hyperoxygenation from breathing enriched normobaric oxygen.

We also have related the measurements with EPR with *in vivo* NIR and also with polarographic transcutaneous oxygen. There have been no reported or observed side effects from the injections of Indian Ink. We have, however found that in some subjects there is up to several weeks after initial injection before the ink becomes responsive to oxygen. We are investigating the basis for this effect; this lack of responsiveness has not been observed in animals or in tumors in human subjects.

While still at an early stage, the oximetry measurements in human subjects have demonstrated the feasibility of the technique, including the capability of carrying out the measurements within the constraints of usual clinical practice. Extensive studies are now being planned for a systematic development and evaluation of EPR oximetry in cancer patients.

Dosimetry

Another major use of clinical EPR is for the purposes of dosimetry [3, 77, 78]. The importance of this area is reflected in the high level of funding that has been made available for its development and the widespread recognition of the unique contributions that can be made by *in vivo* EPR to solve some of the critical problems in this area. As a consequence of the high level of activity that has been supported for this application development of instrumentation and techniques, the technical capabilities for all types of clinical EPR have been significantly advanced, while extensive experience has been gained in the practical aspects of making measurements in human subjects with *in vivo* EPR. The technical/practical advances that will be applicable to all types of clinical applications of EPR include: (i) Development of transportable EPR instruments, which will facilitate the adoption of clinical EPR to bedside and intra-operative applications; (ii) Development of operating characteristics so that it can be used by non-expert operators in any environment; (iii) Features that accommodate routine, minimally perturbing, and rapid measurements with an instrument that is appropriate for use with compromised human subjects; and (iv) Extensive demonstration of the safety and efficacy of EPR measurements in human subjects.

The potential value of the use of EPR dosimetry arises from an unfortunate fact of our current world: the potential for widespread exposures of large numbers of individuals to levels of ionizing radiation that are acutely life-threatening. The exposures could come from accidents (although these are unlikely to result in acutely life-threatening levels of exposures to many people), terrorism, or war. There are treatments available that reduce the severity of the accurate radiation syndrome but these are too complex and too potentially toxic to allow them to be used except if there is a reasonable probability that the subject has received potentially lethal doses of radiation. With a capability to screen large numbers of individuals rapidly, responders could direct into the health care system those who have received potentially life-threatening exposures and allow the rest of the population to know that they do not need acute treatment. This problem could be ameliorated if everyone would wear badges to detect significant radiation exposures, but the consensus is that this is impractical because of failure to comply and potential malfunction or misuse of passive radiation badges such as ones intrinsic to credit cards or driver's licenses. Fortunately, however, it turns out that most of us are wearing a dosimeter, our teeth and finger/toe nails [3, 78]. These have sufficiently stable radiation-induced free radicals to act as passive radiation dosimeters. These potentially can be assessed directly by EPR spectroscopy *in vivo*.

The basis of EPR dosimetry resides in its unique ability to directly measure free radicals. Ionizing radiation generates large numbers of free radicals and while most of these react quickly and disappear in microseconds or less, they can persist if they are generated in materials that have little liquid water and have some systemic organization. The

hydroxyapatite matrix in tooth enamel is such a tissue and it has been established that linearly dose-dependent radiation-induced changes in teeth last millions of years and there serve as excellent dosimeters. The EPR signal arises from radical ions generated from carbonate randomly distributed in the hydroxyapatite. The keratin-rich regions of nails also provide a site where radiation-induced radicals are stabilized. These persist for weeks but are more complex to analyze because mechanical forces also can generate radicals in keratin [79–82].

Currently almost all of the measurements in human subjects for EPR radiation dosimetry have been done using teeth as the dosimetric site (Figure 3). The focus is on the use of the two upper central incisor teeth because of their favorable anatomy (they are relatively flat), the higher probability that they will be present and without major restorations, and the convenience of making measurements in teeth at this position. With the support of BARDA (Biomedical Advanced Research and Development Authority) we are moving forward with the goal of producing an FDA-approved manufacturable dosimeter that can be employed in the field as part of the planned responses to a large radiation event. The characteristics for this instrument include features that will be very useful for all clinical applications including simplified operation, rapid measurement time (less than five minutes), attention to the needs for comfort and accessibility of potentially compromised subjects, rugged and reproducible construction, and full safety features compatible with clinical use. The current status of the developments are indicated by the initial data on dose response of human subjects measured *in vivo*, using patients undergoing total body irradiation (TBI) as the model for radiation effects in teeth of human subjects irradiated in the field. The current resolution of dose in the mouth model using human teeth is about 0.5 Gray standard error of inverse prediction (SEIP). In irradiated subjects, preliminary results indicate that it may be feasible to achieve the same dose resolution, but more data are needed to confirm this. This level of accuracy is more than sufficient for the purpose of initial triage.

The status of *in vivo* nail dosimetry is less advanced but quite promising. Because the radiation-induced signals in nails are weaker than those in teeth, we are assuming that the measurements will need to be made with higher frequency EPR (9 GHz vs. the 1.1 GHz) used for other clinical measurements. Satisfactory measurements of radiation-induced signals in fingernail models have been made at 9 GHz [78]. The challenge for this application is to design and build resonators that limit the microwave field to the nail, avoiding the soft tissue below because the latter would result in non-resonant absorption of the microwave. Considerable progress has been made on such resonators so that useful spectra have been obtained from living fingers (Figure 4). Developments are proceeding to enable the measurements to be made of the radiation-induced signals in the nails of patients undergoing radiation therapy that results in significant doses to their nails.

Conclusion

The recent developments in EPR instrumentation, probes, and methods offer potential opportunities for clinical oximetry and dosimetry. The EPR oximetry is valuable for 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. The use of *in vivo* EPR dosimetry appears to be sufficient for the initial stage of triage in a large-scale radiation event and further improvements are underway, consistent with the goal of becoming an intrinsic part of the response system within 2–3 years.

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Figure 1. Clinical EPR system

(A) Our 1.2 GHz EPR spectrometer using a large-gap permanent magnet and surface loop resonator. Also shown is a moveable bed for patient positioning. (B) Measurement of tumor pO_2 in a head & neck cancer patient using surface loop resonator.

Figure 2. Implantable resonator design

Photograph of a 25-cm-long implantable resonator made of 34-AWG enamel-coated copper wire containing PDMS-encapsulated LiNc-BuO sensor at one end (Probe tip) and coupling loop at the other end. The inset shows an EPR spectrum obtained using the resonator.

Figure 3. *In vivo* tooth dosimetry using deployable EPR dosimeter

(A) 1.15 GHz EPR dosimeter setup. (B) Magnet, modulation coil, and resonator assembly.

(C) Surface-loop resonator (~9-mm diameter loop). (D) Side view of the dosimeter with the person undergoing tooth measurement.

Figure 4. Photographs illustrating the position of fingers in resonators for in vivo nail dosimetry (A) SRA resonator, (B) rectangular TE_{102} cavity aperture resonator (AR), and (C) hemispherical TE_{121} AR.

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 pro-inflammatory, pro-fibrotic, and pro-angiogenic growth factors and cytokines that lead to tissue fibrosis.

Table 2
pO₂ measurements in human tumors measured in vivo with challenge by breathing enriched oxygen.

Number	Cancer	Site	Response to Oxygen	With Air (mmHg)	With Oxygen (mmHg)
1	Melanoma	Knee	Yes	<10	>50
2	Lymphoma	Post. Thigh	Yes	<5	>100
3	Melanoma	Foot	No	Anoxic	Anoxic
4	Melanoma	Scalp	No	Anoxic	Anoxic
5	Sarcoma	Nose	No	~10	~10
6	Melanoma	Scalp & Neck	Yes	~6	~12
7	Melanoma	Scapula	Yes	<1	<5
8	Melanoma	Chest	No	~10	~10
9	Melanoma	Calf	Yes	Anoxic	~3
10	Sarcoma	Post. Thigh	Yes	<3	>10
11	Sarcoma	Post. Thigh	Yes	~1	>5