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Clinical Applications of In Vivo EPR: Rationale and Initial Results

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Abstract. In vivo electron paramagnetic resonance (EPR) has been very useful for studies in animals, and these results suggest that there are some very attractive potential applications in human subjects. In this article, we describe our rationale for the clinical application of in vivo EPR, some of the principal technical challenges, the initial results in human subjects, and our evaluation of the areas where in vivo EPR is likely to play an important clinical role in the near future. The most obvious area of very high potential for clinical applications is tissue oximetry, where in vivo EPR can provide repeated and accurate measurements of tissue pO_2, a type of measurement that cannot be obtained by other techniques. Oximetry is capable of providing clinicians with information that can impact directly on diagnosis and therapy, especially for peripheral vascular disease, oncology, and wound healing. The other area of great immediate importance is the ability of in vivo EPR to measure clinically significant exposures to ionizing radiation after the fact, which may occur due to accidents, terrorist activity, or nuclear war. The results obtained already from human subjects demonstrate the feasibility of the use of in vivo EPR for measurements in human subjects. We anticipate that in vivo EPR will play a vital role in the clinical management of various pathologies in the years to come.

1 Rationale and Our Approach for the Clinical Applications of In Vivo EPR

In vivo electron paramagnetic resonance (EPR) has been applied successfully and extensively to measure important physiologic parameters in animal models such as tissue pO_2, free radicals, pH, perfusion, and redox status [1]. It seems clear that measurements of many of these parameters could be utilized effectively to advance medical care, with the potential to improve significantly diagnosis and therapy. We therefore have undertaken an active research program to determine fully the methodology needed to make such measurements and to establish the usefulness of such measurements.

We believe that the key step for the successful clinical use of in vivo EPR is to establish application(s) where EPR has clear advantages, either by providing unique information or by demonstrating that it can provide information more

accurately, more sensitively, and/or more reliably than can be done by existing technology. We therefore have focused our initial efforts on applications that can most rapidly meet these criteria. The applications that we are pursuing initially are repetitive oximetry and measurement of clinically significant exposures to ionizing radiation. Both of these types of measurements would be valuable for problems that are widely recognized as being of great importance and for which in vivo EPR has some clear advantages over other existing techniques that could be used to try to solve them. We believe that after in vivo EPR is successfully introduced into clinical medicine, it will be quite feasible to introduce other additional uses, which may become equally or even more valuable. This approach is based in part on observations of the way in which magnetic resonance imaging (MRI) was introduced successfully.

2 Potential Challenges and Constraints for the Use of In Vivo EPR in Human Subjects

While the use of in vivo EPR in human subjects is likely to provide major potential benefits, in order to achieve these we need to overcome some significant challenges. We have identified several key steps that need to be carried out in order for in vivo EPR measurements to be carried out successfully and safely in human subjects.

2.1 Develop the Techniques for In Vivo EPR in Ways That Fully Satisfy Regulatory Requirements for Its Safe Use in Human Subjects

Because of the low magnitude of the magnetic field and the types of electromagnetic fields that are employed, these instrumental aspects of in vivo EPR are unlikely to cause problems. The highest potential source of problems is local heat deposition, but it now has been shown that under the usual conditions for measurements in human subjects, the heat deposition will be well within existing FDA regulations [2]. These measurements were made directly in appropriate model tissues in our laboratory under conditions that are similar to those used for clinical measurements. We also have built up considerable empirical evidence that local heating is not likely to be a problem. Studies have been carried out in a number of volunteers making measurements in both the foot (for oximetry) and in teeth in the mouth (for retrospective dosimetry), and in these studies there has been no indication of any problems of local heating.

The need to administer paramagnetic materials for many potential applications, however, raises some potential problems that could be very time-consuming and costly to surmount if the materials have not been previously cleared for use in human subjects. The soluble probes (nitroxides, spin traps, trityl variants) will be excreted fairly rapidly, so the potential for toxicity may be limited to effects that occur quite quickly. It is likely that clearance for their use in human

subjects will be pursued as is typical for new diagnostic agents, but that will require considerable time and effort and because these are not essential for our initial applications, we will leave this for others to pursue. There are some other potential uses for nitroxides [3], so their clearance may occur in that context. It is unlikely that these compounds would be considered a suitable investment for clearance to be pursued solely for their use for in vivo EPR. One of the feasible types of spin traps for nitric oxide, the dithiocarbamates, already is in clinical use as an alcohol avoidance agent and as a chelator for metal toxicity, so it may be feasible to utilize these types of compounds on the basis of their approval for other uses.

The particulate paramagnetic probes used for oximetry are chemically inert, which leads to their long-term retention in the tissues. This facilitates repeated noninvasive measurements of tissue pO_2 as desired for weeks to several years. However, for obtaining approval for their routine use in human subjects, long-term testing is necessary to demonstrate that this will not lead to undesirable side effects. This would be especially time-consuming and costly. Therefore, our strategy for oximetry is to use India ink, which already is in use in humans [4]. India ink has been used as an external and internal marker in clinics for the last several decades with no reported toxicity. The use of India ink as an oximetry probe has allowed us to take a safe and quick step towards clinical application of EPR oximetry for peripheral vascular disease [5, 6] and for measurements of oxygen in tumors.

An alternative approach is to enclose the paramagnetic materials in a biocompatible material with a permeable membrane (such as used in microresonators) [7] to avoid direct contact with the tissue or blood. This type of arrangement may make it feasible to obtain permission for their use in human subjects without the extensive data needed for their direct administration. While such invasiveness may be difficult to justify for many applications, this should be quite practical for measurements in tumors. This application also is made more feasible by the usual need to make a surgical biopsy to confirm the diagnosis of cancer; during such procedures the microresonators could readily be implanted.

There are no such limitations, of course, when we do not need to add paramagnetic materials, as is the case in retrospective dosimetry where we follow radiation-induced EPR centers in teeth or following free radical intermediates of drugs or normal processes.

2.2 Overcome the Potential Limitations of Sensitivity and Depths at Which the Measurements Can Be Made

Because of high nonresonant dielectric loss of the radiofrequency wave due to the high water content in biological samples; the penetration depth of the most commonly applied frequency of 9–10 GHz (X-band) is less than 1 mm. We have focused on the development of spectrometers working at a lower frequency (1200 MHz), which has made it possible to conduct in vivo measurements on a

variety of small animals and isolated organs, although the depth of penetration is still rather limited for studies in large animals and human subjects. At this frequency, for many applications the sites that can be probed usually need to be within 10 mm of the surface. Within this limit there are a number of very promising applications that can be developed, and our initial studies have focused on such applications, as described in the following sections.

It also should be noted that there are some very feasible ways to increase the depth at which the measurements could be made. The choice of the solution will depend on the nature of the measurement that is sought. One method is to reduce the frequency to well below 1200 MHz to increase the depth that the microwaves will penetrate, although this may decrease the sensitivity [8]. Another approach is to use implantable microresonators [7], which will allow highly sensitive measurements to be made deep in tissues.

2.3 Accommodating Human Subjects in the In Vivo EPR Spectrometer and Compensating for Physiological Movements

The adaptation of the instrument(s) for accommodating human subjects involves straightforward engineering developments, especially because the demands for the strength of the magnetic field are quite modest (450 G or less). The magnet system should have sufficient distance between the poles to readily and comfortably accommodate patients who are likely to have significantly compromised physical capabilities. In addition to good magnetic field homogeneity, a sufficiently strong and homogeneous modulation field is required for clinical measurements.

Measurements in living systems, including human subjects, can be degraded due to voluntary and physiological motions which can induce noise and/or cause the movement of the paramagnetic materials into less homogeneous regions of the main magnetic field or modulation field. Some of these effects can be minimized by the use of automatic tuning and automatic matching [9, 10]. It also is necessary to make it feasible for the human subjects, who may be ill and/or weak, to be comfortably placed and restrained. Fortunately, such restraint is necessary for many types of measurements and treatments, so we have been able to utilize solutions developed by others for this purpose. In particular, we obtained very useful advice from our colleagues in Radiation Oncology, who routinely need to place patients repetitively and comfortably into positions that will enable them to control the sites that are irradiated.

Using these principles, a clinical EPR facility on the basis of a 400 G permanent magnet (Sumitomo Special Metals, Torrance, CA) with a gap of 50 cm between poles has been developed with appropriate procedures to position the subject comfortably but effectively restrained to minimize noise due to motion [11]. In order to position the subject in the magnet, a special gantry (made from nonmagnetic material) has been constructed that can be moved on rails into the magnet. Figure 1 shows a subject sitting comfortably with his foot in the magnet permitting pO_2 measurements in regions of critical importance for

Fig. 1. Measurements can be performed while the patient is comfortably seated in front of the magnet. The image on the left shows a volunteer's foot in place in the EPR magnet, while measurements of pO_2 are being made. The other images show a volunteer's foot during EPR and TcO_2 measurements. TcO_2 electrodes were placed on the dorsal side of the foot (center) and the EPR measurements were done at the metatarsal head on the plantar surface of the foot (right).

following the course of peripheral vascular disease. Measurements also can be made in the prone position on the same gantry. We also have designed a chair that will fit between the poles of the magnet, enabling subjects to be in a sitting position within the magnet; this will be especially useful for measurements in the mouth.

3 Initial Results in Human Subjects

3.1 Measurements of Oxygen in the Foot, Suitable for Enhancing Treatment of Peripheral Vascular Disease

The use of in vivo oximetry in the clinic is clearly the most likely initial extensive clinical application of in vivo EPR. It has the potential to provide sensitive and accurate direct measurements of tissue pO_2 in the foot. Such measurements will provide direct data on oxygen status in tissues that are at risk for diabetic ulcers and subsequent complications. These data will be unique and potentially clinically valuable because the key pathophysiological changes in these patients are linked to poor oxygenation of tissues. Currently there is no way to measure this directly, and instead clinicians must rely on clinical signs and symptoms, measurements of blood flow (which are related to oxygenation of tissues in only a complex and variable manner), or transcutaneous oxygen (TcO_2) measurements which, as noted below, are rather indirect and sometimes give erroneous measures of the actual pO_2 at the sites of interest [12, 13].

Currently TcO_2 is the only method used clinically to determine tissue pO_2 for the management of the ulcerated diabetic foot. It has been advocated as a means to determine the likelihood of ulcers to heal, to select an appropriate site for amputation, and for the measurement of the effectiveness of hyperbaric oxygen treatment [13]. However, its use remains quite problematic. TcO_2 measurements have a standard deviation of daily measurements of ± 5 mm Hg and, in practice,

a change of 10–20 mm Hg is needed to be significant, so this technique is used at the limits of its reliability [12]. More fundamentally, it is not clear how the measurements of the TcO_2 technique relate to the actual tissue oxygenation, as the measurement involves the heating of the underlying tissue to 42–44 °C and the oxygen level is measured by an oxygen electrode at the skin surface. In addition, the oxygen is measured by a process in which the oxygen is continuously consumed. It is difficult to get reliable TcO_2 measurements over thick skin because skin thickness impedes oxygen diffusion. Therefore, TcO_2 measurements are made on the dorsum of the foot with the assumption that this reflects surface oxygenation in all regions of the foot, especially the plantar surface where the ulcers occur.

We believe that direct tissue oximetry with in vivo EPR will improve assessment of the risk of complications and significantly advance the capability to monitor the effectiveness of therapies over time. As the first step to determine the validity of this approach, we have initiated experiments to establish the feasibility of using EPR oximetry to make measurements in the feet in healthy volunteers, using India ink as the oxygen-sensitive material because of its acceptability for use in human subjects [4–6]. The India ink used in these experiments is prepared under strictly controlled conditions with components that are already in use in various pharmaceutical preparations. The reproducibility of this ink and its sensitivity to oxygen in different batches has been thoroughly examined and is found to be very satisfactory [14]. The ink (20 μl) was injected under the first metatarsal head, the site of greatest risk for patients with peripheral vascular diseases, and a series of pO_2 measurements were carried out over the last year (Fig. 2).

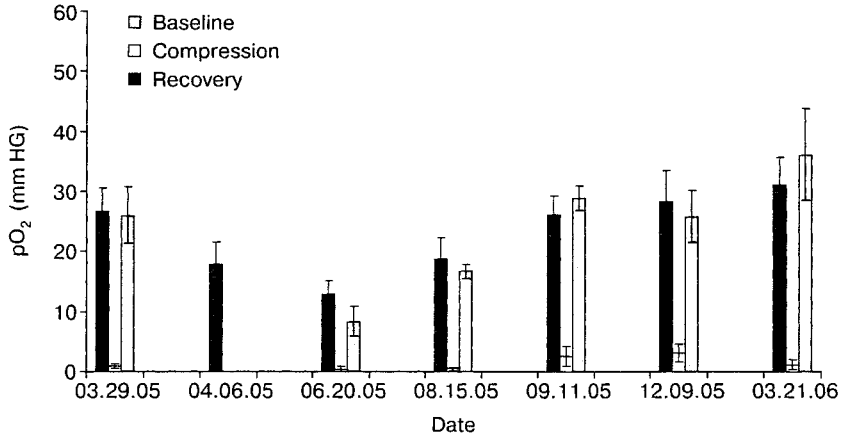

Fig. 2. Tissue pO_2 of the metatarsal head of a healthy volunteer. India ink was injected on March 22, 2005, and tissue pO_2 was measured over a period of 5 min during compression and 10 min each during baseline and after compression was released (recovery). The foot temperature was maintained at 37 °C with a warm air blower in all the measurements. Mean ± S.D.

While the data obtained from repeated measurements over long periods of time are likely to be very valuable, it also would be desirable to be able to probe more dynamic aspects of the status of the foot. We therefore have employed two different procedures to confirm the ability of EPR oximetry to detect the acute changes in tissue pO_2 caused by perturbations. It is likely that the response of tissue pO_2 to these perturbations could be used as a valuable parameter to determine the status of the peripheral vascular disease, measuring parameters related to both delivery and utilization of oxygen in the affected tissues. The first procedure is local compression of the thigh, impeding the delivery of blood to the foot. This provides data on the decrease of pO_2 once the blood flow is impeded (which is a measure of utilization) and then the recovery after the compression is released (which is measure of both delivery and utilization). The results obtained in a healthy volunteer with compression tested over one year are summarized in Fig. 2. Results indicate a consistent decrease in tissue pO_2 during compression, and the pO_2 returning to baseline (initial) values after the compression was released. There was, however, more variation of the baseline over time than was expected. While it is possible that this is due to random variations, a more systematic cause, such as a temporary change in the calibration of the material, cannot be ruled out. Further studies on this important aspect are underway.

The second perturbation that we are studying is the response to breathing increased amounts of oxygen (Fig. 3). This is a very mild procedure that could be employed in virtually all subjects regardless of the status of their health. In addition to the EPR measurements of India ink placed under the plantar surface at the metatarsal head of the first toe, we also measured tissue pO_2 using TcO_2 at the dorsal side of the foot (Fig. 4). The measurements made with EPR were at the site that is most susceptible to diabetic ulcers due to peripheral vascular

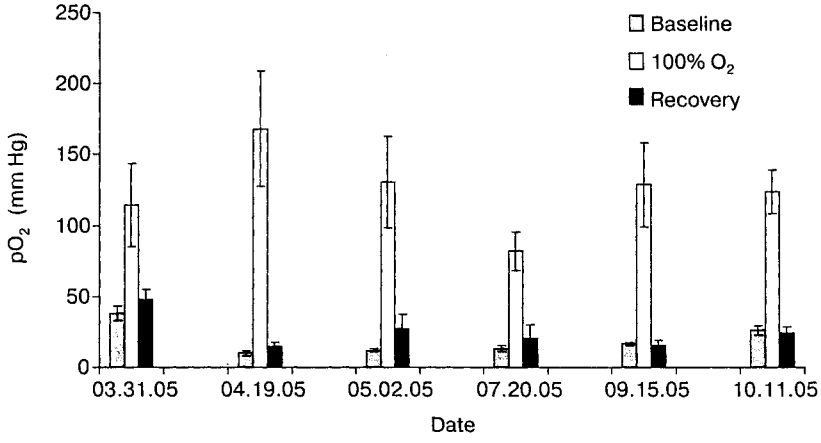

Fig. 3. Tissue pO_2 of the metatarsal head of the healthy volunteer in response to breathing 100% oxygen for 10 min. Baseline and recovery data were collected for 10 min with the volunteer breathing room air. The foot temperature was maintained at 37 °C with a warm air blower in all the measurements. Mean ± S.D.

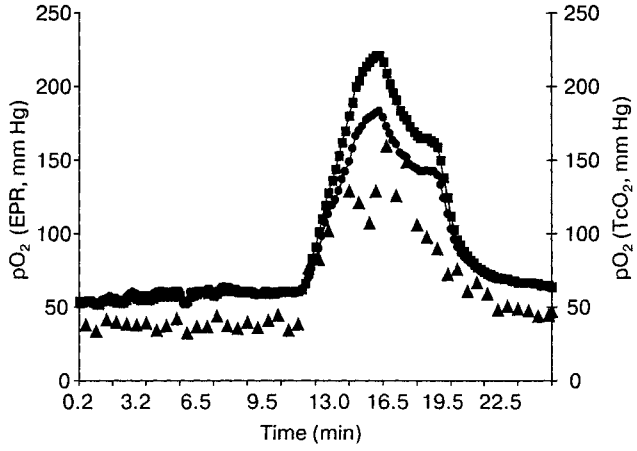

Fig. 4. Tissue pO_2 of the metatarsal head of a healthy volunteer in response to breathing 100% O_2 (▲). TcO_2 measurements were done at the first interosseous space (■) and between the 4th and 5th toes (●) of the dorsal surface. Three EPR spectra of 10 s each were averaged to obtain one data point. The TcO_2 data are recorded at an interval of 10 s each.

disease. The measurements of TcO_2 were made at the usual site of clinical TcO_2 measurements, which was chosen because the technique cannot be used on the plantar surface where the skin is thick. Therefore, one needs to assume that the measurements on the dorsal surface are reflective of the conditions at the site of real interest. The results indicate that the dynamics of the two types of measurements were similar, but the baseline pO_2 measured by TcO_2 was higher. This is not surprising because the sites are physiologically quite different and as part of the usual method for measuring TcO_2, the skin under the electrode was heated to 42–44 °C.

These initial results indicate the feasibility of using in vivo EPR oximetry to make repeated measurements and also to follow acute perturbations. Such data should allow clinicians to evaluate the status of disease and the response to therapy. The interpretation of the dynamic data in terms of complex processes, such as oxygen utilization and delivery, will be challenging but possible. As clinical experience builds up, the repeated pO_2 measurements and the dynamic data should be very useful for classifying patients empirically as to the status of their disease and the effects of disease progression and therapy.

3.2 Repeated Measurements of Oxygen in Tumors To Follow the Effects of Disease Progression and Responses to Therapy

The role of oxygen in tumors is important and complex. Because of their rapid growth and disorderly blood supply, tumors tend to have regions of low oxygen tension that sometimes result in necrosis. The presence of hypoxia in tumors has significant implications because the response to radiation drops when the pO_2

is less than 15–20 mm Hg, and tumors tend to become more aggressive when hypoxic [15–17]. The situation is further complicated by the fact that therapy can alter the pO_2 in tumors in a complex and time-dependent pattern. Typically following irradiation the pO_2 in tumors decreases and then, depending on the type of tumor, the pO_2 increases in many tumors. As cells die, the pO_2 in the remainder of the tumor may increase due to decreased numbers of cells that are consuming oxygen [17]. Chemotherapy also can affect the tumor pO_2 in similarly complex ways. Empirically the pO_2 in tumors is often the most accurate predictor of the response to radiation therapy [18].

We hypothesize that if one could repeatedly measure the pO_2 in tumors, especially during the time when the tumors are being treated, it would be possible to modify the timing and type of treatment to increase the response to therapy by delivering it at times of maximum tumor pO_2. This would increase the therapeutic ratio, because the normal tissues usually have pO_2 that are well above the levels that affect the response to therapy, and therefore they would not sustain more damage from the timing of the therapy.

Clinical studies of these hypotheses are underway in our laboratory. The clinical research is in its early stages. Measurements are being made in patients with readily accessible tumors in order to establish the procedures and make observations on the pO_2 of tumors and how it may change with time and/or treatment. Figure 5 shows the first measurement in a human subject of pO_2 in a tumor, a melanoma, using EPR oximetry. The tumor was located at the distal tip of the leg, following an amputation several inches below the knee. India ink (about 40 μl) was injected in the tumor and baseline pO_2 and the response to 100%

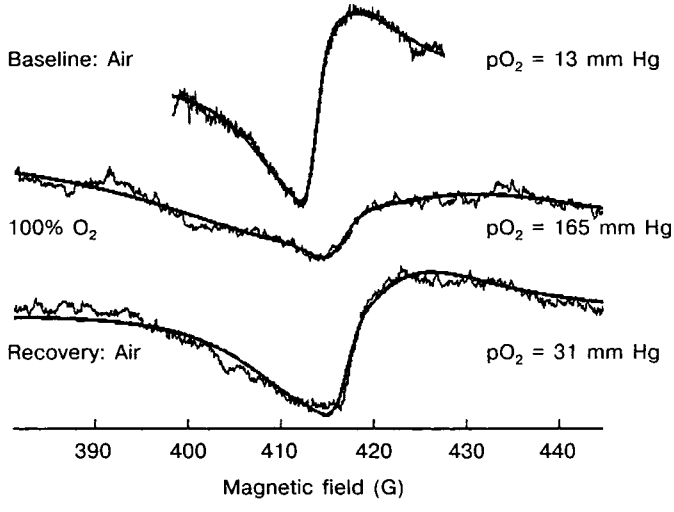

Fig. 5. EPR spectra and tissue pO_2 within a melanoma lesion (volunteer breathing air) and response to breathing 100% O_2. Measurements with air and 100% were averaged for 2.5 and 5 min respectively, and the mean interval between measurements was approximately 8 min.

O_2 breathing was measured. The increase in inhaled oxygen led to a dramatic increase of the pO_2 in the lesion, raising it by more than a factor of 10 from a level of potential radiobiological hypoxia. The tumor subsequently was excised, and the location of the India ink in the excised tumor was confirmed.

3.3 Determination of Radiation Dose after the Fact by Measurement of EPR Signals in Teeth In Vivo

The radicals generated by ionizing radiation in teeth can persist for a very long time and ex vivo measurements have been widely used to determine the radiation dose after the fact [19–22]. With the development of in vivo EPR, it has become possible to make the measurements without removing the teeth. Such measurements would be extremely useful for dealing with the very significant problem of estimating the occurrence of clinically significant exposures to ionizing radiation from acts of terrorism or accidents. There are few if any other physical methods that can make such a measurement after the fact, and therefore there has been a high degree of interest and support from federal agencies for the development of in vivo EPR dosimetry.

We have successfully designed resonators that are effective and comfortable for use within the human mouth. Figures 6 and 7 illustrate the resonators placed on a molar (shown using the model for a human head that we employ to facilitate developments) and on incisors in a volunteer.

While we have previously shown that L-band EPR can make sensitive and accurate measurements of dose in isolated teeth, it is vital to show that sensitive measurements also could be made within the mouth. Figure 8 summarizes a recent experiment that confirms this capability. We made measurements of an irradiated tooth while it was isolated from lossy tissue (extra oral) and again when placed within a volunteer's mouth at the site of a missing tooth (intra oral). The same resonator was used for both measurements and each were acquired in 4.5 min (averaging 90 three-second scans) with the same settings of the EPR spectrometer. As

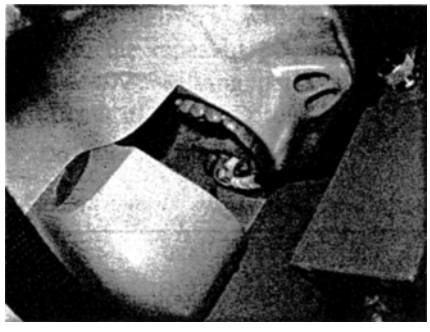

Fig. 6. Resonator for molars installed on an irradiated human molar inserted into the "jaw" of an artificial head. Figure adapted from ref. 23.

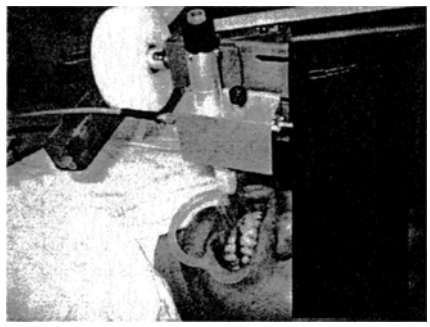

Fig. 7. Resonator for incisors installed in the mouth of a volunteer. Figure adapted from ref. 23.

shown in Fig. 8, the parameter that determines the ability to resolve dose, which is the signal-to-noise (S/N) ratio, the sensitivity of the measurement increased by a factor of 3 (the amplitude of the signal decreased by about half, but the noise decreased by more than a factor of 5). We tentatively have concluded that the latter is due to the suppression of the dominant type of noise (termed microphonics) by the presence of the oral tissues. While repeated measurements have been consistent with this result, more extensive studies are planned, but if this result is fully reproducible, then there will be a very significant gain in the sensitivity of the technique as we go from isolated human teeth to measurements in the mouth.

Another preliminary study was undertaken with a volunteer who has a missing molar to observe the EPR signal in vivo and to gain experience with the reproducible positioning of the resonator in the mouth. All measurements were made using the whole-body clinical EPR magnet, an EPR resonator specifically designed for measurements of molars, and a resonator holder with 3-dimensional

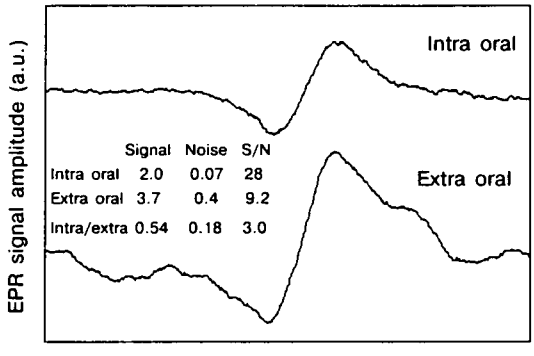

Fig. 8. S/N ratio obtained on the same sample, in the same resonator, when placed inside the mouth of a volunteer (upper trace) versus the same sample with the resonator and sample in air ("extra oral", lower trace). The values of S/N are shown on the plot; the noise was dominated by microphonics.

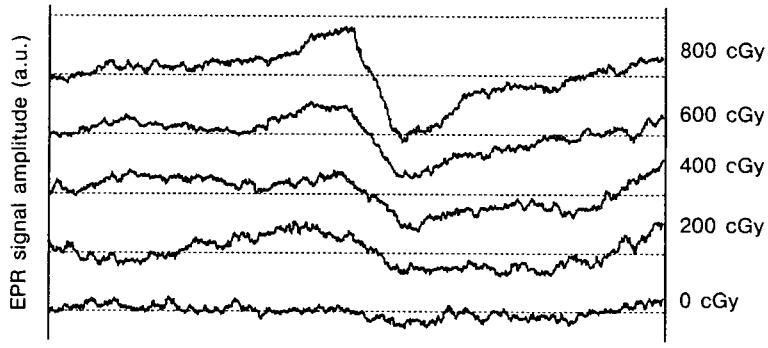

Fig. 9. In vivo dosimetry measurements were carried out using a denture made for a molar tooth that was placed in a natural location in the mouth of a volunteer. This tooth was serially irradiated up to 800 cGy of additional dose at 200 cGy intervals. At each dose, data was collected over 4.5 min and averaged. As the dose increases, the progressively larger signal amplitude is apparent.

fine adjustment capabilities. Measurements were made with the volunteer lying in a supine position. A removable denture was made so that a single irradiated molar could be positioned within the mouth and measured repeatedly as the dose was incrementally increased from 0 cGy (background) to 800 cGy by in vitro irradiations. Procedures were developed to isolate the tooth of interest from nearby lossy tissue, such as the cheeks and tongue, using common dental instruments and techniques. For each dose, a set of 90 spectra was acquired and averaged to increase the S/N ratio. This acquisition required 4.5 min, though it is likely that this time can be reduced. The instrumental parameters used in this study were similar to those used in previous in vitro studies, and include a scan width of 30 G, Zeeman modulation amplitude of 3.5 G, and an incident microwave power of 100 mW. The spectra acquired at these 5 doses are shown in Fig. 9. The dose dependence of the signal amplitude is apparent. Refinement of our techniques for the placement of the resonator, minimization of noise sources that contribute to the baseline drift, and incorporation of a reference standard are currently underway and will lead to improved dosimetric accuracy and precision.

We anticipate that the in vivo dosimetry method described here is likely to be able to determine doses as low as 50–150 cGy and up to more than 3000 cGy. This dose range extends down to radiation doses that are unlikely to cause significant acute symptoms, through the dose range where immediate medical intervention may be very helpful, and also into the dose range where no treatment is likely to be effective. Thus, this technique has the potential for use to screen large numbers of people after potential exposure to clinically significant radiation for effective categorization into treatment plans.

4 Speculations on the Future for the Use of In Vivo EPR in Human Subjects

The use of EPR for retrospective or after-the-fact dosimetry on the basis of measurements of teeth in situ for triage of a population that has potentially been exposed to significant amounts of ionizing radiation seems very probable. It currently appears to be gaining status as the method of choice for such measurements, and because of the threat of terrorism, there is widespread support for the implementation of this capability. Initially the instrumentation used for these measurements is likely to be similar to that used for the present experiments. But there are some potential ways to reduce the size and weight of the magnets needed for these measurements, which would further broaden the applicability of the technique.

The immediate medical applications of in vivo EPR are likely to focus on oximetry, especially where there is a high potential for having an immediate significant impact on the care of patients. The two areas that seem most likely are repetitive measures of oxygen in the foot for the management of peripheral vascular disease and measurements of oxygen in tumors to enhance individualized therapy.

In particular, the use of EPR oximetry for managing peripheral vascular disease should be especially useful in providing an objective measure of the impact of treatment on the fundamental parameter related to the pathophysiology, the amount of oxygen available for tissues at risk of ischemic damage. If this application proves to be beneficial in terms of showing a relationship of the measurements to improved outcomes for the patients, then it is likely to become part of the standard care for most patients with diabetic peripheral vascular disease.

The other area where oximetry has a good probability of becoming part of standard clinical care is for the measurement of tumor pO_2. This is important because the response of tumors to therapy, especially radiation therapy but also chemotherapy, is very sensitive to the concentration of oxygen in the tumor. Therefore, this information could be very useful in enhancing the efficacy of radiotherapy by scheduling treatments at times of optimal tumor oxygenation. With the existing sensitivity available with the oximetry technique, it is possible to carry out pO_2 measurements in peripheral tumors located not deeper than 10 mm from the skin surface. As illustrated in Fig. 5, we have initiated a project to establish the methodology needed to make valid measurements of tumor pO_2 in human subjects under conditions that are compatible with the constraints of clinical practice and comfort of patients. Our goal is to make measurements of tumor pO_2 during the course of radiation therapy and use this information to enhance the therapeutic outcomes. It also should be possible to make measurements in deeper tumors using implanted resonators.

In the near future we anticipate making measurements of oxygen in wounds. This could have an important role in the clinical management of wounds, as the oxygen concentration appears to be a very important factor for wound healing. These studies are likely, at least initially, to utilize some of the materials that

currently are placed within wounds to monitor their healing, making it feasible to use any oxygen sensing paramagnetic material because the material would be enclosed within a gas permeable biocompatible material. This will enable clinicians to monitor the status of oxygen within the wounds so that intervention could be implemented promptly if the conditions for healing become suboptimal [6].

There is a reasonable probability that eventually in vivo EPR also will be used for measurements of free radicals and several aspects of pharmacology; measurements of redox metabolism also are potentially possible in human subjects. There are a number of other types of measurements, especially of biophysical parameters that may be effectively applied in the future [1]. Such measurements are likely to occur only after in vivo EPR is introduced into the clinic for the uses noted above.

5 Summary and Conclusions

In summary, tissue oximetry and after-the-fact dosimetry appear to be the most prominent and immediate clinical applications of in vivo EPR. EPR dosimetry based on radiation-induced changes in teeth is likely to become the method of choice for after-the-fact determination of clinically significant radiation doses. The ability of EPR oximetry to make repeated measurements provides a very important capability that can be effectively used for peripheral vascular disease and tumor therapy. In vivo EPR will likely become a valuable tool for many clinical applications in years to come, with an expanding list of applications based on a number of different parameters that in vivo EPR can measure with particular effectiveness.

Acknowledgments

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