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Forum: Oxidative Stress Status

ACCURATE AND SENSITIVE MEASUREMENTS OF pO_2 IN VIVO USING LOW FREQUENCY EPR SPECTROSCOPY: HOW TO CONFER BIOCOMPATIBILITY TO THE OXYGEN SENSORS

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Abstract—Within the last few years, there has been a significant amount of progress using EPR oximetry, which has resulted in the availability of instrumentation and paramagnetic materials capable of measuring pO_2 in tissues with an accuracy and sensitivity comparable to or greater than that available by any other method. While the results obtained with EPR so far indicate that criteria for the measurements of pO_2—such as accuracy, sensitivity, repeatability, and noninvasiveness—can be met, some of the paramagnetic materials with optimum spectroscopic properties (i.e., strong simple signals which are appropriately responsive to changes in pO_2) may have some undesirable interactions with tissues, causing reactions with and/or losing responsiveness to oxygen. In this paper, several approaches are discussed, such as encapsulation procedures, which can result in the availability of oxygen-sensitive materials in a suitable configuration for long-term studies (absence of toxicity and preservation of the responsiveness to oxygen). © 2000 Elsevier Science Inc.

Keywords—EPR, ESR, In vivo, Oxygen, Encapsulation, Free radicals

INTRODUCTION

Oxidative stress primarily occurs in tissues as the result of an inappropriate oxygen environment for the cells. Small differences (decreases or increases) in the physiological partial pressure of oxygen (pO_2) can induce stress and/or the formation of reactive species with deleterious effects. In studies on oxidative stress, there is more and more recognition that there is a need for systems that can detect very subtle variations of pO_2 in tissues, especially in living systems, in order to predict the relevance of pathophysiological situations. Although the measurement of oxygen is a key factor for understanding oxidative stress, there is a lack of methodologies that are able to report continuously, noninvasively, and accurately the pO_2 in tissues. In this paper, we describe the principles of EPR oximetry, the types of oxygen sensors, and the recent advances to enhance the biocompatibility of these sensors. It is essential to demonstrate that the variations of pO_2 measured are related to physiological or pathophysiological processes, and are not related to disturbances coming from the presence of the oxygen sensor inside the tissue.

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IN VIVO EPR OXIMETRY AND OXYGEN SENSORS

The term *EPR oximetry* encompasses a number of distinct techniques [1]. One class of techniques relies on the oxygen dependence of the rates of metabolism of EPR-sensitive probes (e.g., nitroxides). Other methods rely on the paramagnetic properties of molecular oxygen since oxygen has two unpaired electrons and is therefore effective in relaxing other paramagnetic species. Measurements that depend on T_1 and T_2 provide direct evidence of the local

Table 1. Stability of Several Paramagnetic Compounds When Implanted in Gastrocnemius Muscle of Mice

Paramagnetic material	Stability in vivo	Reference
Lithium Phthalocyanine	< 3 d	[17]
India ink	> 4 months	[18]
Fusinite	> 4 months	[4]
Typical heated charcoal	< 1 d	[12]
Nitroxides	Few min	[11]
Aldrich activated carbon decolorizing 16155-1	> 3 months	[6]
Aldrich activated carbon darco 27809-2	< 7 d	[6]
Merck charcoal activated GR 99-002186/0250	< 7 d	[6]
Merck charcoal activated extra pure 99-021841/1000	< 3 d	[6]
Fluka activated charcoal 05105	< 1 d	[6]
Strem chemicals carbon powder 93-0601	< 2 d	[6]
EM Science charcoal wood CX0670-1	> 4 months	[6]
Carlo Erba charcoal powder 332658	> 3 months	[6]

concentration in oxygen. By far, the most common method is the measurement of the broadening of the principal hyperfine line (related to T_2). The line broadening is due to Heisenberg spin exchange between unpaired electrons of the probe and paramagnetic oxygen. The physical description is well established for soluble paramagnetic compounds (such as nitroxides). However, the characterization of the physical processes leading to the broadening of particulate materials is still being investigated [2]. Particulate materials offer a more sensitive response than do the soluble materials. Because of their intrinsic high sensitivity, variations of less than 1 mm Hg can be measured directly in vivo after implantation of such materials acting as oxygen reporters. Several paramagnetic probes have been described and used in vivo as oxygen sensors: lithium phthalocyanine [3], charcoals such as fusinite [4] or gloxy [5], activated charcoals [6], and synthetic carbohydrate chars [7]. The measurement in vivo can be performed using low-frequency EPR spectrometers. At low magnetic field, the wavelength to be used is compatible with deep penetration into the tissues, and EPR spectra can be recorded in living systems. A principal goal to further develop EPR oximetry is to achieve the following characteristics: increased sensitivity (ability to detect very subtle changes of pO_2), localization (ability to make the measurement from a defined volume), repeatability, rapidity, noninvasiveness, and biocompatibility [8].

SPECIFIC PROBLEMS OF THE METHODOLOGY

While the results obtained so far with EPR oximetry indicate that we can meet many of the criteria noted above, there are also a number of potential limitations of the current capabilities. We have found that some of the paramagnetic materials with optimum spectroscopic properties (i.e., strong simple signals which are appropriately responsive to changes in the pO_2) may have some undesirable interactions with tissues, causing reactions with and/or losing responsiveness to oxygen. These problems are due to a loss of paramagnetism and/or loss of responsiveness to oxygen.

Loss of paramagnetism

Nitroxides are quickly converted into diamagnetic hydroxylamines, and the metabolism of the nitroxides is oxygen-dependent [9]. Therefore, it was previously suggested that the monitoring of the nitroxide decrease was a possible tool for measuring the pO_2 both in vitro and in vivo. Changes in nitroxide kinetics were also used in specific circumstances to detect the formation of oxidative radicals, thus reflecting the oxidative stress status of the tissue [10]. In fact, the decrease in the EPR signal coming from a specific tissue in vivo can be due to many other factors (the strongest dependence is from the washout of the compound and is related to the perfusion of the tissue; other mechanisms such as diffusion inside the tissue or necrosis of a tissue could also affect the decay of the EPR signal) [11]. Another way to use nitroxides as pO_2 reporters is to use their line width or the super hyperfine splitting (observed with some nitroxides such as carbamoyl-proxyl) as indicators of their environment. For this approach it is, of course, more convenient to get a stable signal from the sensor, and therefore to protect the nitroxide from this metabolism or from rapid washout. The inclusion of nitroxides inside microcapsules or microdevices that are still permeable to oxygen could in this way enhance their usefulness as oxygen sensors in tissues.

Loss of responsiveness to oxygen

There are several experiments that show the relative instability of the oxygen sensitivity under specific conditions. As illustrative examples, we present in Table 1 data on the stability of the oxygen response of several paramag-

netic materials in the gastrocnemius muscles of mice. When a paramagnetic material has an EPR line width that is dependent on the pO_2, one of the simplest ways to evaluate its usefulness as an in vivo marker of pO_2 consists of injecting a small amount of this material into a leg muscle of mice or rats; after equilibrium is reached in the tissues, hypoxic conditions can be easily obtained by reversible restriction of the blood supply. To demonstrate the stability in the responsiveness, one can repeat these experiments over days, weeks, or months. In this context, instability in vivo means that EPR line widths in vivo tend to have the same values in both normal and hypoxic tissues. At the end of the experiment the paramagnetic material should be removed from its site after the sacrifice of the animal and the calibration curve (line width as a function of the pO_2) should be repeated to determine whether the calibration curve was modified. Both types of experiments (in vivo modification of the line width following a clear change in pO_2 and possible change of the calibration curve) are needed to determine the stability of the sensor to its environment. As shown in Table 1, the loss of oxygen sensitivity of the paramagnetic material can occur within a few days for several materials, potentially limiting their usefulness in this tissue.

Concerning the loss of oxygen-sensitivity with time, several considerations should be emphasized. (i) The loss of responsiveness to oxygen is material-dependent and tissue-dependent. For example, lithium phthalocyanine loses the responsiveness to oxygen within a few days in gastrocnemius muscle of mice, but has a stable response for months in the brain or in the spinal cord of rats. Similar findings were made using different types of charcoal. (ii) The time needed to reach the equilibrium for a sensor in its environment is variable from one material to another and from one tissue to another (this phenomenon should not be confused with the loss of responsiveness to oxygen). (iii) There is a need to demonstrate the stability for a specific batch of material; caution should be used when extrapolating results that were established using another batch of oxygen sensors. (iv) The loss of responsiveness to oxygen seems to be the consequence of a modification in the environment of the particle. Although these phenomena are not yet fully understood, it is likely that the instability of the response is related to "chemical" changes at the surface of the particles and/or physical limitation in the accessibility of oxygen. The first event generally occurs rapidly, and the change in the calibration also can be demonstrated in vitro (e.g., in tissue homogenates). In this case, the response will be quickly lost in vivo. The second type of event could be related to the deposit of proteins or other factors after a reaction inside the tissue: such biological components can form a small capsule. As the sensitivity of the response is strongly dependent on the collisions with oxygen, any barrier can lead to a change in the responsiveness to oxygen. In this case, the loss of responsiveness will occur progressively in vivo. Such reactions were also described for some biosensors implanted in tissues. Some biosensors rely on a catalytic reaction for which oxygen is important; some biosensors progressively lose their activity because a tissue reaction occurs, leading to the formation of a capsule surrounding the sensor, which makes a barrier to oxygen [13].

CONCEPT OF BIOCOMPATIBILITY

The biocompatibility of an implanted material can be defined as follows. The performance of the material should not be affected by the host, and the host should not be affected by the implanted material. The potential modifications decreasing the performance of the oxygen sensors include both loss of paramagnetism and/or loss of responsiveness to oxygen. The reactions affecting the host include inflammatory and/or toxic reactions in tissues. It appears quite possible that the embedding of oxygen-sensitive paramagnetic materials in biopolymers that already are acceptable for use in humans would facilitate their approval for use in human subjects. The development of appropriate coating/embedding capabilities appears to be an effective means to reduce some of these potentially important limitations.

Some of the potential beneficial effects of the coatings include: (i) decreasing the probability of the paramagnetic materials having deleterious biological reactions either directly or by stimulating processes such as cytokine release or specific immune response; (ii) decreasing the probability of the paramagnetic materials being altered by the biological system such that they have a decreased signal intensity (due to a decrease in the number of spins and/or an increase in the intrinsic line width) and/or a change in their responsiveness to oxygen (either a loss of responsiveness or a change in the calibration curve); (iii) stabilizing small particles in tissues, preventing their unwanted aggregation or movement; (iv) enhancing their spectroscopic characteristics.

The coating material to be used in order to stabilize the responsiveness of the oxygen sensor as well as to avoid a deleterious tissue reaction should possess the following characteristics. (i) The material should have a good permeability to oxygen. (ii) The coating material should be stable in tissues and should isolate completely the sensor in order to preserve its configuration as long as needed for the studies. The polymers generally used for designing a drug delivery system are biodegradable (e.g., co-polymer of polylactide and polyglycolide). For the present purpose, there is a need to maintain the system in its initial configuration, and consequently to use a polymer without degradation within this time period. (iii) The material should be known to have good tolerance in tissues, and preferably already be accepted for human

use. (iv) The material should be sufficiently versatile to lead to a reproducible and uniform coating.

APPLICATIONS: HOW TO CONFER THE BICOMPATIBILITY TO EPR OXYGEN SENSORS?

Soluble materials (e.g., nitroxides)

In order to avoid a direct contact of the nitroxide with the surrounding tissue, and consequently lose the paramagnetism, several approaches such as the inclusion of nitroxides in implants or the microencapsulation of the nitroxides in liposomes or microspheres have been described [14,16,17]. Interestingly, one of the first studies of in vivo EPR oximetry described the use of an oxygen-permeable capsule containing a nitroxide in solution implanted in the peritoneal cavity of a mouse [14]. Using such a capsule has several advantages: this physical barrier avoids the interaction between the nitroxide solution and the tissue components responsible for the bioreduction of the nitroxide into hydroxylamines; this plastic container also permits the exact localization of the region where oxygen tension is to be determined; and this system allows the use of a nonaqueous solvent with higher oxygen solubility, consequently increasing the sensitivity of the measurement. Oximetry using nitroxides is based on EPR line broadening caused by Heisenberg exchange between molecular oxygen dissolved in solution and the nitroxide. There is a direct relationship between the EPR line exchange broadening and the radical-radical collision rate [15]. The exchange rate, and consequently the EPR line width, is proportional to the solubility of oxygen in the solvent. In this pioneering experiment, the authors used an oxygen-permeable capsule in a plastic TPX, a methylpentene polymer. The nitroxide used was 15N-Tempone dissolved in light paraffin oil, and wax was used to seal the capillaries of PTX. This method had high sensitivity and the sensor remained intact during the period necessary for the measurement.

Another approach based on the same principle was carried out at the microscopic scale. In this approach, the nitroxide was encapsulated in liposomes [16] or proteinaceous microspheres [17]. Glockner et al. described the preparation of large unilamellar liposomes containing positively charged deuterated nitroxides [16]. Compared to unencapsulated nitroxides, these encapsulated nitroxides had several advantages. (i) The stability of the nitroxide encapsulated in liposomes was higher than free nitroxide after administration by intramuscular injection. (ii) This system provided stable levels of free nitroxides in vivo, which is needed for accurate oxygen measurements based on line broadening. However, as the nitroxides are dissolved in an aqueous phase inside the liposomes, the sensitivity of the nitroxide to changes of pO_2 remains low for in vivo applications. The approach of Liu et al. [17] overcame the problem of poor nitroxide sensitivity. In this study, proteinaceous microspheres filled with nitroxides dissolved in an organic liquid were synthesized with high intensity ultrasound. Due to the increase of sensitivity of the line width to oxygen because of the higher solubility of oxygen in organic solvents, reliable measurements of pO_2 were obtained in vivo. A potential concern is the release of the organic solvent inside the tissues as a potential source of toxicity. These authors suggested that the same approach using inert solvents such as perfluorocarbons might solve this problem. This system also protected the nitroxide from bioreduction. However, these microspheres are metabolized over extended periods, and the measurements of pO_2 using this system will be valid only for short-term studies. The particulate probes might be better for extended periods such as weeks or months.

Encapsulation of particulate oxygen sensors

All types of oxygen-sensitive particles (e.g., lithium phthalocyanine and charcoals) could potentially benefit from a convenient coating. Up to now, because of the low availability of lithium phthalocyanine, only studies describing the coating of carbonaceous particles have been reported.

Inks and synthetic inks

Since india ink is very well tolerated by living tissues [18,19], our group tried to prepare a special ink for in vivo EPR applications. The ink consisted of a carbon-based material (fusinite or a carbohydrate char) with optimal EPR properties, dispersed in a liquid solution containing gums or suspending agents [12]. Such a coating could have several advantages: preventing aggregation of small particles (<1 μm) due to electrostatic attraction; improving the tolerance by the tissues and decreasing immunological responses; facilitating systemic injection instead of a local injection with appropriate sized particles, and allowing liver oximetry (accumulation occurs in Kupffer cells). In the case of oxygen-sensitive materials that lose their oxygen sensitivity with time, the coating may prevent this loss of sensitivity to the pO_2. Suspensions of the paramagnetic material were made in water solutions containing 3% arabic gum. Very small particles were obtained using different homogenization systems. To select particles of different sizes, centrifugation and filtration on calibrated filters were used. No significant differences were observed among the calibration curves for the different water suspensions

of carbon materials, which had either been coated, contained small particles, or contained large particles (within the range of size used in this study). Although no significant difference was observed in vitro in the pO_2 sensitivity for coated or uncoated materials, we observed that several coatings had a dramatic effect on the pO_2 sensitivity in vivo [12]. The coating with arabic gum preserved the pO_2 sensitivity of the fusinite particles. The pO_2 sensitivity (measured by observing the line width change in unrestricted or blood-restricted muscles) remained unchanged during the 6 weeks of the experiments. The chars used in that study lost their oxygen sensitivity in vivo and the coatings using these hydrosoluble polymers were unable to prevent this loss of oxygen sensitivity. The use of small particles of fusinite (300 nm diameter) coated with arabic gum made intravenous administration possible with eventual accumulation in the liver. The uncoated fusinite was toxic when administered by iv, due to the large size of the particles (apparently the particles formed aggregates that were trapped in the capillaries of the lungs). Although we demonstrated at that time the feasibility of synthesizing inks with optimal EPR properties, the approach of using hydosoluble polymers did not preserve the responsiveness to oxygen of unstable chars. Therefore, we have assumed that the preparation of sensors with a deposit of an insoluble thin film of oxygen-permeable biopolymer on its surface can preserve the responsiveness to pO_2 inside the tissue.

Film coatings of sensors

Many techniques of microencapsulation exist and are used in pharmaceutical technology. As a starting point, we used well-established methods that were already described in the literature for coating charcoals. Charcoal, due to its large adsorption capacity, can be used in the treatment of drug intoxication. The technique, called "charcoal hemoperfusion," relies on the passage of the blood through a column containing charcoal particles in order to remove the drugs or xenobiotics causing the intoxication. In this system, charcoal particles are directly in contact with blood elements. In order to overcome problems associated with platelet removal, excessive blood damage, and/or the release of fine carbon particles into the blood stream, activated charcoal has been successfully coated with many biocompatible polymers. These include albumin-collodion, nylon and collodion, cellulose derivatives, polyhydroxyethyl methacrylate, silicone, polyethyleneglycol, and polyacrylate-polymethacrylate. For our initial studies, we selected two charcoals which have the oxygen-sensing properties required for EPR oximetry combined with a tendency to lose responsiveness to oxygen when placed in tissues [6]. We prepared different batches of coated materials, varying the amount of pyroxylin (cellulose nitrate) used as the coating material [20]. Particles of defined sizes were selected using mechanical sieves to produce materials in increments of 25 μm, ranging from smaller than 25 μm to larger than 175 μm; the smallest and the largest particles were tested both as uncoated and coated materials. There were no significant differences in the calibration curve (line width as a function of pO_2) between the uncoated and coated materials. The thin layer film made by this cellulose derivative is sufficiently permeable to oxygen, which allows equilibrium with the external medium. Finally, we evaluated the performance of the coated particles as oxygen sensors by inducing hypoxia in the muscle of mice injected with charcoals and we repeated the experiments for 2 months to determine the reproducibility and the stability of the EPR line width of the paramagnetic material. As shown in Fig. 1, the uncoated charcoal used in this study lost its responsiveness to oxygen within 1 week; the responsiveness to changes of pO_2 decreased 1 week after the injection of the charcoal, and there was a complete loss of responsiveness by 9 d after the injection. Using the same animal model as for uncoated materials, we found no loss in the responsiveness to oxygen over 2 months (time of observation) when a sufficient amount of coating material was used (20 to 40% w/w). Using a lower content in pyroxylin, the responsiveness to oxygen was not preserved. Using a higher amount of pyroxylin (more than 40% w/w), we observed the formation of films that were difficult to handle and inject in animals. Coated particles can thus be used in long-term studies where an accurate measurement of the pO_2 in tissues is necessary. We concluded that these results demonstrate that an appropriate film coating on the surface of charcoals (i.e., using pyroxylin) is able to preserve the responsiveness to oxygen of paramagnetic materials used for in vivo EPR oximetry [20].

Biocompatible implants as oxygen reporters

Another approach of EPR oximetry is based on devices directly implanted into living tissue. This includes the preparation of very small implants and the development of coating/membranes for the attachment to insertable probe resonators. We are following strategies already used in the design of biosensors, especially devices requiring the use of biocompatible membranes permeable to oxygen. Most of them use thin silicone tubing. Other biocompatible oxygen-permeable materials could also be used for that purpose. One application we developed relied on the preparation of biocompatible silicon implants containing fusinite [21]. Finely ground fusinite was mixed with polydimethysiloxane oil and silicon paste, placed in a syringe, and extruded

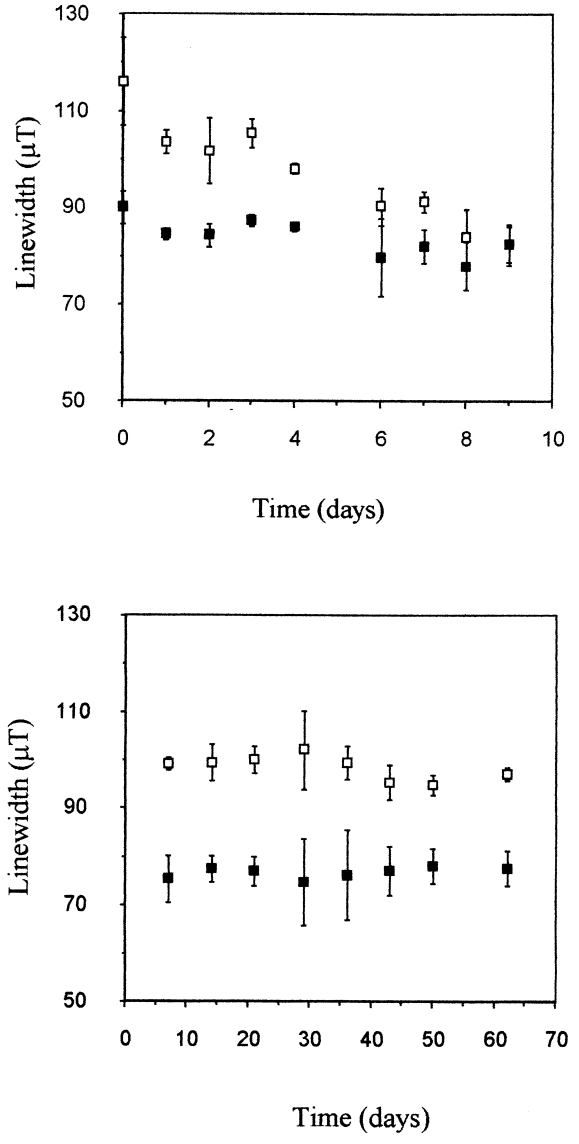

Fig. 1. Effect of the coating using pyroxylin on the responsiveness of a charcoal to changes of pO_2 in vivo. *Top*: Change of the responsiveness to oxygen in vivo observed on a typical unstable charcoal. The EPR line width was recorded using an L-Band spectrometer on anesthetized mice before (□) and after (■) the restriction of the blood flow in the muscle. Note the decrease in the responsiveness to changes of pO_2 one week after the injection, and the complete loss of responsiveness 9 d after the injection. *Bottom*: The same charcoal was coated using 30% (w/w) pyroxylin. Note that the coating preserved the responsiveness to oxygen for more than 2 months.

in a Silastic (Dow Corning, Midland, MI, USA) medical grade tubing. Small pieces of ± 2 mm were cut and sealed. The calibration curves of the fusinite in a slurry compared to those of the silicon implant indicated a larger slope value, indicating a higher sensitivity to pO_2 for the silicon implants than for the fusinite slurry. The reasons for this increase in sensitivity are not yet clear. In contrast to the soluble paramagnetic compounds (i.e., nitroxides) where the EPR line width directly depends on the oxygen concentration, the EPR line width of particles is more independent of oxygen concentration, but more dependent on the partial pressure of oxygen. Therefore, it is likely that this particular observed behavior is not linked to the higher oxygen solubility in silicone than in aqueous phase, but rather is due to changes at the surface of the charcoal particles, leading to a change in sensitivity.

CONCLUSIONS AND PERSPECTIVES

The need for a special coating and/or embedding of paramagnetic materials is increasing. The interest for such studies started when it appeared that some paramagnetic materials were unstable in biological media and that a special coating can enhance their usefulness by stabilizing their responsiveness. Moreover, with the success of the in vivo EPR applications in living animals, specifically oximetry, it was apparent that it would be very useful to apply this technology in humans. Before this technique can be applied to humans, extensive research must be done to determine which paramagnetic compounds are biocompatible. The alternative is to incorporate these probes into biocompatible implants, a strategy that would greatly reduce the time required for approval for its use in human subjects. In this manuscript, we have presented several possible strategies. We should emphasize that this area of research is very new, and that the recent progress made in pharmaceutical technology should greatly help in finding suitable coatings of paramagnetic materials [22,23].

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REFERENCES

[1] Swartz, H. M.; Glockner, J. F. Measurement of oxygen by EPRI and EPRS. In: Eaton, G. R.; Eaton, S. S.; Ohno, K., eds. *EPR imaging and in vivo EPR.* Boca Raton, FL: CRC Press; 1991: 261–290.

[2] Clarkson, R. B.; Ceroke, P. J.; Norby, S. W.; Odintsov, B. Water interactions in porous carbohydrate chars: multi-frequency DNP, EPR, and NMR studies. *International workshop on techniques and biomedical applications of in vivo EPR and PEDRI* (abstr). 1999:29.

[3] Liu, K. J.; Gast, P.; Moussavi, M.; Norby, S. W.; Vahidi, N.; Walczak, T.; Wu, M.; Swartz, H. M. Lithium phthalocyanine: a probe for electron paramagnetic resonance oximetry in viable biological systems. *Proc. Natl. Acad. Sci. USA* **90:**5438–5442; 1993.

[4] Vahidi, N.; Clarkson, R. B.; Liu, K. J.; Norby, S. W.; Wu, M.; Swartz, H. M. In vivo and in vitro EPR oximetry with fusinite: a new coal-derived, particulate EPR probe. *Magn. Reson. Med.* **31:**139–146; 1994.

[5] James, P. E.; Grinberg, O. Y.; Goda, F.; Panz, T.; O'Hara, J. A.; Swartz, H. M. Gloxy: an oxygen-sensitive coal for accurate mea-

surement of low oxygen tensions in biological systems. *Magn. Reson. Med.* **38:**48–58; 1997.
[6] Jordan, B.; Baudelet, C.; Gallez, B. Carbon-centered radicals as oxygen sensors for in vivo electron paramagnetic resonance: screening for an optimal probe among commercially available charcoals. *MAGMA* **7:**121–129; 1998.
[7] Clarkson, R. B.; Odintsov, B.; Ceroke, P.; Ardenkjaer-Larsen, J. H.; Fruianu, M.; Belford, R. L. EPR and DNP of char suspensions: surface science and oximetry. *Phys. Med. Biol.* **43:**1907–1920; 1998.
[8] Swartz, H. M.; Clarkson, R. B. The measurement of oxygen in vivo using EPR techniques. *Phys. Med. Biol.* **43:**1957–1975; 1998.
[9] Chen, K.; Glockner, J. F.; Morse, P. D.; Swartz, H. M. Effects of oxygen on the metabolism of nitroxide spin labels in cells. *Biochemistry* **28:**2496–2501; 1989.
[10] Utsumi, H.; Takeshita, K.; Miura, Y.; Masuda, S.; Hamada, A. In vivo EPR measurement of radical reaction in whole mice. Influence of inspired oxygen and ischemia-reperfusion injury on nitroxide reduction. *Free Radic. Res.* **19:**S219–S225; 1993.
[11] Gallez, B.; Bacic, G.; Goda, F.; Jiang, J. J.; O'Hara, J. A.; Dunn, J. F.; Swartz, H. M. Use of nitroxides for assessing perfusion, oxygenation, and viability of tissues: in-vivo EPR and MRI studies. *Magn. Reson. Med.* **35:**97–106; 1996.
[12] Gallez, B.; Debuyst, R.; Liu, K. J.; Goda, F.; Walczak, T.; Demeure, R.; Taper, H.; Swartz, H. M. Small particles of fusinite and carbohydrate chars coated with aqueous soluble polymers: preparation and applications for in vivo EPR oximetry. *Magn. Reson. Med.* **40:**152–159; 1998.
[13] Park, H.; Park, K. Biocompatibility issues of implantable drug delivery systems. *Pharm. Res.* **13:**1770–1776; 1996.
[14] Subczinski, W. K.; Lukiewicz, S.; Hyde, J. S. Murine in vivo L-Band ESR spin-label oximetry with a loop-gap resonator. *Magn. Reson. Med.* **3:**747–754; 1986.
[15] Hyde, J. S.; Subczynski, W. K. Spin-label oximetry. In: Berliner, L. J.; Reuben, J., eds. *Biological magnetic resonance. Spin labeling: theory and applications.* New York: Plenum Press; 1989: 399–425.
[16] Glockner, J. F.; Chan, H. C.; Swartz, H. M. In vivo oximetry using a nitroxide-liposome system. *Magn. Reson. Med.* **20:**123–133; 1991.
[17] Liu, K. J.; Grinstaff, M. W.; Jiang, J.; Suslick, K. S.; Swartz, H. M.; Wang, W. In vivo measurement of oxygen concentration using sonochemically synthesized microspheres. *Biophys. J.* **67:**896–901; 1994.
[18] Goda, F.; Liu, K. J.; Walczak, T.; O'Hara, J. A.; Jiang, J.; Swartz, H. M. In vivo oximetry using EPR and india ink. *Magn. Reson. Med.* **33:**237–245; 1995.
[19] Goda, F.; Bacic, G.; O'Hara, J. A.; Gallez, B.; Swartz, H. M.; Dunn, J. F. The relationship between partial pressure of oxygen and perfusion in two murine tumors after X-ray irradiation: a combined gadopentetate dimeglumine dynamic magnetic resonance imaging and in vivo electron paramagnetic resonance oximetry study. *Cancer Res.* **56:**3344–3349; 1996.
[20] Gallez, B.; Jordan, B.; Baudelet, C. Microencapsulation of paramagnetic particles by pyrroxylin in order to preserve their responsiveness to oxygen when used as sensors for in vivo EPR oximetry. *Magn. Reson. Med.* **42:**193–196; 1999.
[21] Gallez, B.; Debuyst, R.; Liu, K. J.; Demeure, R.; Swartz., H. M. Development of biocompatible implants of fusinite for in vivo EPR oximetry. *MAGMA* **4:**71–75; 1996.
[22] Mäder, K. EPR and controlled drug delivery. Something to offer in both directions. *International workshop on techniques and biomedical applications of in vivo EPR and PEDRI* (abstr). 1999: 21.
[23] Gallez, B. Packaging of paramagnetic materials in oximetry and other applications. *International workshop on techniques and biomedical applications of in vivo EPR and PEDRI* (abstr). 1999: 31.

ABBREVIATIONS

EPR—Electron Paramagnetic Resonance
pO_2—partial pressure of oxygen
w/w—weight/weight