Applied Magnetic Resonance
https://doi.org/10.1007/s00723-021-01358-7

Applied
Magnetic Resonance

REVIEW

# Oxygenation Status in Normal Tissues, Pathological Tissues and Malignant Tumors: A $pO_2$ Database Based on Electron Paramagnetic Resonance (EPR) Oximetry Measurements

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Received: 15 February 2021 / Revised: 10 May 2021 / Accepted: 18 May 2021


## Abstract

The purpose of this manuscript is to provide an overview of methods that are termed EPR oximetry and to describe how these techniques have been successfully applied to characterize oxygenation status in normal and pathological tissues as well as in malignant tumors. Values in oxygenation status are presented for the following tissues: brain, kidney, liver, heart, skeletal muscle, skin, and grafts. We report basal $pO_2$ values recorded in physiological conditions, values that may considerably vary depending on the anesthesia used. We also present the evolution of the oxygenation under pathological or ischemic states, under oxygen-enriched breathing challenge, and after pharmacological intervention. Because tumor hypoxia is a key detrimental factor in tumor progression and resistance to radiation therapy, EPR oximetry has been increasingly being used not only to characterize hypoxic tumor phenotypes but also to monitor changes in oxygen status after therapeutic interventions (i.e. oxygen or carbogen breathing, modulators of perfusion, inhibitors of oxygen consumption rate, irradiation). EPR oximetry has also been used to qualify or disqualify other oxygen imaging (PET, MRI) biomarkers. Overall, this paper presents a large comprehensive database on $pO_2$ values recorded in different tissues and in different physiological conditions using EPR oximetry.

Special issue: 85th birthday of Harold M. Swartz.

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Published online: 11 June 2021

## 1 Introduction

Hypoxia has been long recognized as a crucial factor in many disorders and in treatment response or failure, a statement that justified considerable efforts to develop non-invasive oximetry techniques. The purpose of this manuscript is to provide an overview of methods that are termed electron paramagnetic resonance (EPR) oximetry [1–4] and to describe how these techniques have been applied to characterize oxygenation status in normal and pathological tissues as well as in malignant tumors.

## 2 Principles and Modalities

For biomedical or clinical researchers who are not familiar with the technology, it is worth briefly introduce this spectroscopic method. EPR or equivalently ESR (electron spin resonance) is a magnetic resonance method which detects species containing unpaired electron(s). Although the principles of EPR and NMR are comparable, differences in physicochemical properties of the resonant species (unpaired electrons vs nuclei with net spin) lead to differences in the techniques that are used to record the spectra or build an image. Three differences can be emphasized: the resonance condition (frequency/magnetic field ratio), the need for paramagnetic compounds, and the very short relaxation times of the electron spins [1]. The greatest difference between NMR and EPR arises because the gyromagnetic ratio of an unpaired electron is much larger than that of a proton. As a consequence, the resonance frequency/magnetic field ratio is 28 GHz/T for the electron while it is 42.5 MHz/T for the proton. Consequently, standard EPR spectrometers operate at higher frequencies and lower fields than conventional NMR spectrometers. Most standard commercial EPR spectrometers operate at 9–10 GHz with a magnetic field sweeping around 0.3 T. At this frequency, non-resonant absorption of the electromagnetic radiation (microwave frequencies) by aqueous samples presents a serious problem, thus limiting the sample size to a thickness of less than 1 mm. Larger aqueous samples (tissues from animals or humans) can be studied only by reducing the operating frequency [5]. Most in vivo oximetry studies have been performed at 1 GHz with a depth of sensitivity of about 1 cm which is ideal for studies in mice or rats. The use of systems operating at lower frequencies extends the sensitive depth to sampling volume but unfortunately reduces the signal-to-noise ratio of the measurements. The second major difference between EPR and NMR relies on the need for paramagnetic species. Except for melanin [6, 7], compounds with unpaired electrons are present in insufficient amounts to be detected by low-frequency EPR. Therefore, a paramagnetic substance has to be introduced into the tissue. Identifying stable paramagnetic species has been a significant factor in the successful development of in vivo EPR oximetry. The third difference relies on the relaxation times of paramagnetic species that are much shorter (nanoseconds) compared to nuclei analyzed by NMR where the relaxation times typically are milliseconds. This has two major consequences. First, in vivo EPR spectra are obtained through continuous wave (CW) experiments

in most centers with the notable exception of developments made at Bethesda (Krishna's group) [8] and Chicago (Halpern's group) [9]. The second consequence of short EPR relaxation times is that EPR imaging requires magnetic gradients that are at least one order of magnitude greater than those used in MRI.

Now, how to measure oxygen using EPR? Molecular oxygen possesses two unpaired electrons and is therefore paramagnetic. However, no EPR spectra have been reported from oxygen dissolved in fluids or tissues at physiological temperature. Fortunately, indirect EPR methods exist. Most EPR oximetry methods rely on the relaxing properties of molecular oxygen which decreases the EPR relaxation times for other paramagnetic species [10]. The enhancement of relaxation rates increases with the concentration of oxygen over a wide range of oxygen tensions. Measurements that depend on $T_1$ and $T_2$ of EPR spin probes introduced in a biological system provide a direct indication of the oxygenation status. The most common method is to use the broadening of the EPR linewidth (LW) that is inversely related to $T_2$. Whatever the type of oxygen sensor material used, in practice, a given paramagnetic material is calibrated in terms of the effect of oxygen on the LW. When introduced in tissues, the measurement of the linewidth of the sensor can be directly interpreted in terms of the oxygenation in the vicinity of the probe.

Two classes of paramagnetic compounds are used as EPR oxygen sensors: soluble materials and insoluble particulate materials. For soluble paramagnetic materials, the LW varies linearly with increasing concentration of oxygen [10]. Soluble probes have the advantage of diffusing throughout a tissue. Two types of structures are interesting within the class of soluble oxygen sensors: the nitroxides and the triarylmethyl (or trityl) radicals. Nitroxides are stable free radicals where the unpaired electron is delocalized between nitrogen and oxygen. The effect of oxygen on the linewidths of these compounds is fairly modest but sufficient for several applications. The relative changes in LW induced by oxygen are much larger for perdeuterated nitroxides that possess a very narrow LW. Perdeuterated nitroxides have been used in vitro for measuring oxygen consumption by cells [11, 12] or in vivo for oximetry purposes [13, 14]. It is also possible to use nitroxides with a resolved superhyperfine structure [15]. To increase the sensitivity of the oxygen measurement, strategies have been used to encapsulate nitroxides in lipophilic environments [16]. Consistent with the Smoluchowski equation, as oxygen is more soluble in lipophilic environments than in water, an increase in sensitivity can be achieved using these systems. Paramagnetic nitroxides are rapidly metabolized into diamagnetic hydroxylamine resulting in a loss of EPR signal. Of note, the kinetics of bio-reduction of nitroxide may also be used to assess the redox state of tissues [17–21]. For oximetry purposes, trityl radicals are more appropriate because of the narrowness of their LW (often less than 10 μT). As the signal intensity is inversely proportional to the square of the EPR LW, the sensitivity of detection is particularly convenient for in vivo applications [22–25]. Moreover, the oxygen-induced broadening of the LW is relatively larger compared to nitroxides. Finally, the narrow LW is particularly suitable to increase the spatial resolution in EPR imaging. The soluble EPR sensors present the inconvenient to be rapidly cleared from a tissue, requiring multiple administrations if longitudinal oximetry studies are needed.

For solid particulate paramagnetic materials, the relationship between the EPR linewidth and the partial pressure of oxygen can be much more complex. The biggest advantage in using particulate materials is that most of them that were identified as oxygen sensors share an extremely large broadening per unit of $pO_2$, exceeding that of soluble radicals by several orders of magnitude [1, 2, 26, 27]. The interaction with oxygen responsible for the line broadening depends on the structure of the particulate material. For lithium phthalocyanine and derivatives, the oxygen sensitivity is critically dependent on the crystal form [28–32]. Oxygen has been shown to migrate in the channels present in the crystals [29, 32]. In carbonaceous materials (chars, coals, carbon blacks) [33–36], the carbon-centered free radicals are stabilized over large aromatic ring clusters. Generally, these materials contain several types of paramagnetic centers characterized by their own oxygen sensitivity and LW which generally lead to a non-linear evolution of the LW as a function of the $pO_2$ [37]. Still, several carbon materials present a remarkably high sensitivity to oxygen at low partial pressure making them particularly suitable for measuring ischemic processes or tumor hypoxia. Another very important feature shared by most particulate oxygen sensors is their remarkable inertness and stability in tissues. As a consequence, once introduced inside a tissue, these materials can report oxygenation status from the same site over a very long period of times (months or years). To ensure the biocompatibility of the oxygen sensors and their possible use in humans, carbon materials may be formulated in stable pharmaceutical excipients that also improve the rheological behavior of the suspensions and limit the diffusion inside tissues [38–40]. Another strategy has been to encapsulate the oxygen sensors in inert biopolymers (such as polydimethylsiloxane or Teflon) to limit the interactions between the cells and the paramagnetic materials while keeping the free diffusion of oxygen inside the implants [41–47]. The use of several point probes together with appropriate magnetic field gradients allows to separate the EPR signals coming from different paramagnetic sensors and therefore to record oxygen readings from different sites at the same time (multi-site EPR oximetry) [48–51].

## 3 Normal Tissues and Associated Pathologies

EPR oximetry has been applied in a broad range of tissues under different physiological or pathological conditions. The tables presented in this paper review a very large number of studies. This list is rather comprehensive. However, it does not include reports where $pO_2$ values were not clearly stated. As already emphasized, the nature of the probe used for the measurement (soluble vs particulate) as well as the technique used for recording the EPR signal (single-point, multi-site, imaging) may influence the reading of the oxygenation status. Moreover, the nature of the anesthesia regimen may also have a profound impact on the hemodynamics and oxygenation status [52–56]. Therefore, these parameters have been systematically recorded in building these Tables.

*Brain* EPR oximetry has been used in a large series of studies to monitor brain oxygenation (Table 1). While lipophilic nitroxides have been designed to cross the blood–brain barrier [57, 58], the variation in LW observed with these probes were

**Table 1** Oxygenation status reported in the brain using EPR oximetry

| Technique | Oxygen sensor | Anesthesia | Oxygenation under Basal/Physiological conditions | Challenge or Pathology | Oxygenation under challenge or pathological conditions | References |
|---|---|---|---|---|---|---|
| Spectroscopy | LiPc | Awake | 35 mmHg | 10.5% $FiO_2$ | 15 mmHg | [54] |
| | | | | 75% $FiO_2$ | 65 mmHg | |
| | | Isoflurane | 25 mmHg | | | |
| | | Ketamine/Xylazine | 20 mmHg | | | |
| | | Pentobarbital | 17 mmHg | | | |
| Spectroscopy | LiPc | Ketamine/Xylazine | | 30% $FiO_2$ | 16 mmHg | [59] |
| | | | | 15% $FiO_2$ | 8 mmHg | |
| | | | | 10% $FiO_2$ | 6 mmHg | |
| Spectroscopy | LiPc | Isoflurane | | 21% $FiO_2$ | 38 mmHg | [55] |
| | | | | 33% $FiO_2$ | 45 mmHg | |
| | | | | 50% $FiO_2$ | 53 mmHg | |
| | | Ketamine/Xylazine | | 100% $FiO_2$ | 56 mmHg | |
| | | | | 21% $FiO_2$ | 3.5 mmHg | |
| | | | | 33% $FiO_2$ | 5.6 mmHg | |
| | | | | 50 $FiO_2$ | 7 mmHg | |
| | | | | 100% $FiO_2$ | 8.8 mmHg | |
| | | Pentobarbital | | 21% $FiO_2$ | 9 mmHg | |
| | | | | 33% $FiO_2$ | 14 mmHg | |
| | | | | 50% $FiO_2$ | 19 mmHg | |
| | | Chloralose/Urethane | | 21% $FiO_2$ | 13 mmHg | |
| | | | | 33% $FiO_2$ | 18 mmHg | |
| | | | | 50% $FiO_2$ | 21 mmHg | |
| | | | | 100% $FiO_2$ | 27 mmHg | |

**Table 1** (continued)

| Technique | Oxygen sensor | Anesthesia | Oxygenation under Basal/Physiological conditions | Challenge or Pathology | Oxygenation under challenge or pathological conditions | References |
|---|---|---|---|---|---|---|
| | | Halothane | | 21% $FiO_2$ | 17 mmHg | |
| | | | | 33% $FiO_2$ | 23 mmHg | |
| | | | | 50% $FiO_2$ | 29 mmHg | |
| | | | | 100% $FiO_2$ | 32 mmHg | |
| Multi-site spectroscopy | LiPc | Isoflurane | 33 mmHg | MCAO | | [60] |
| | | | | Ischemic core | 1 mmHg | |
| | | | | Penumbra | 11 mmHg | |
| Multi-site spectroscopy | LiPc | Isoflurane | 38 mmHg | 2 h post MCAO | | [50] |
| | | | | Ischemic core | 10 mm Hg | |
| | | | | Perifocal area | 13 mmHg | |
| Spectroscopy | LiPc | Isoflurane | 31 mmHg | 90 min post MCAO in penumbra | | [61] |
| | | | | 30% $O_2$ inspired | 12 mmHg | |
| | | | | 70% $O_2$ inspired | 17 mmHg | |
| | | | | 95% $FiO_2$ | 34 mmHg | |
| | | | | 100% $FiO_2$ | 38 mmHg | |
| Multi-site spectroscopy | LiPc | Isoflurane | 25 mmHg | 60 min post MCAO | 15 mmHg | [62] |
| Spectroscopy | LiPc | Isoflurane | | $VEGF^{+/+}$ | 40 mmHg | [63] |
| | | | | $VEGF^{\delta/\delta}$ | 40 mmHg | |
| | | | | $VEGF^{188/188}$ | 40 mmHg | |
| | | | | 10% $FiO_2$ | 20 mmHg | |
| | | | | 95% $FiO_2$ | 80 mmHg | |
| Spectroscopy | LiPc | Isoflurane | 33 mmHg | 1 h post Methamphetamine (8 mg/kg) | 24 mmHg | [64] |

**Table 1** (continued)

| Technique | Oxygen sensor | Anesthesia | Oxygenation under Basal/Physiological conditions | Challenge or Pathology | Oxygenation under challenge or pathological conditions | References |
|---|---|---|---|---|---|---|
| Spectroscopy | LiPc | Isoflurane | | Hypertensive stroke-prone rats | | [65] |
| | | | | 12 weeks-age control | 34 mmHg | |
| | | | | 16 weeks-age control | 26 mmHg | |
| | | | | 12 weeks-age UCAO | 37 mmHg | |
| | | | | 16 weeks-age UCAO | 13 mmHg | |
| Spectroscopy Implantable resonator | LiPc | Isoflurane | 30% O2 inspired<br>41 mmHg | 15% $FiO_2$<br>Carbogen inspired | 30 mmHg<br>70 mmHg | [66] |
| Multi-site spectroscopy | LiPc | Isoflurane | | 30% $FiO_2$<br>15% $FiO_2$ | 40 mmHg<br>24 mmHg | [67] |
| Implantable resonator | | | | 30 min post clot stroke | 25 mmHg | |

too small to provide reliable oxygenation status [57]. Still, these probes are interesting to provide information on the redox status of the tissue [58]. In most studies, lithium phthalocyanine (LiPc) has been used to provide quantitative estimates of the brain oxygenation [50, 54, 55, 59–67]. Several features of LiPc are favorable for their application in the brain. The high spin density and the narrowness of the EPR signal makes it possible to use single crystals that are stereotactically implanted in the area of interest. Moreover, the linear dependence evolution of the LW as a function of the $pO_2$ makes this probe able to provide oxygen estimates over a large range of oxygen concentration as observed in the brain. In the earliest studies demonstrating the ability of EPR oximetry to evaluate brain oxygenation [54, 55, 59], it was observed that both the anesthesia regimen as well as the fraction of inspired oxygen ($FiO_2$) had a considerable impact on brain oxygenation. Among the anesthetics tested, inhalation of isoflurane kept the highest $pO_2$ in the brain [55]. On the contrary, the administration of ketamine/xylazine or pentobarbital led to a dramatic drop in brain oxygenation. Since then, most studies were carried out under isoflurane anesthesia. Depending on the studies, basal $pO_2$ values reported in the brain ranged from 25 to 41 mmHg [50, 54, 55, 60–67]. As the main application, EPR oximetry has been used to characterize dynamically the changes in brain $pO_2$ over time during ischemic stroke after middle cerebral artery occlusion (MCAO) [50, 60–62]. Multi-site spectroscopy has shown its unique ability to monitor at the same time evolution of oxygenation in the ischemic core, in the penumbra, and in the contralateral hemisphere. In the context of fundamental studies on the role of VEGF isoforms on neuronal migration, metabolic and oxygenation studies were carried out on transgenic mice. Namely, normal vascular densities, normal oxygenation, and metabolic parameters were found in transgenic mice $VEGF^{\delta/\delta}$ with deletion of the Hypoxia Response Element (HRE) in the VEGF gene and in mice $VEGF^{188/188}$ expressing exclusively the $VEGF_{188}$ isoform [63]. Other studies have evaluated the impact of methamphetamine exposure on striatal $pO_2$ [64]. These authors found that $pO_2$ was significantly reduced 24 h after administration of a single dose of methamphetamine and that continual exposure exacerbates the condition [64]. Oxygenation was also measured chronically in the white matter (WM) in spontaneously hypertensive stroke-prone rats: the rats first experienced an increase in WM $pO_2$ from 9 to 12 weeks followed by a dramatic decrease in WM $pO_2$ to near hypoxic conditions during weeks 13–16 after unilateral cerebral artery occlusion (UCAO) [64]. More recently, EPR oximetry with a deep-tissue multi-site oxygen-sensing probe (implantable resonator) was used to monitor temporal changes in cerebral $pO_2$ simultaneously at four sites in a rabbit model of ischemic stroke induced by embolic clot [66, 67].

*Kidney* EPR oximetry has been used to measure tissue oxygen tension simultaneously in the kidney cortex and outer medulla in vivo in mice [68]. In this study, $pO_2$ in the cortex region was higher compared to that in the outer medulla (Table 2). An intravenous injection of endotoxin (LPS) resulted in a sharp drop in $pO_2$ in the cortex and an increase in the medulla region, resulting in a transient period of equal $pO_2$ in both regions. The authors also found that pretreatment of mice with NG-monomethyl-L-arginine prevented these changes in tissue $pO_2$ [68]. Another study analyzed dynamically the evolution of $pO_2$ in both the renal cortex and medulla in rats during a renal ischemic-reperfusion injury process [69]. The $pO_2$ in the cortex

**Table 2** Oxygenation status reported in the kidneys using EPR oximetry

| Technique | Oxygen sensor | Anesthesia | Oxygenation under Basal/ Physiological conditions | Challenge or Pathology | Oxygenation under challenge or pathological conditions | References |
|---|---|---|---|---|---|---|
| Spectroscopy | LiPc | Ketamine | 15 mmHg | Aortic ligation | 4 mmHg | [28] |
| Spectroscopy | LiPc | Ketamine/Xylazine | Cortex 22 mmHg<br>Medulla 15 mmHg | LPS | Cortex 19 mmHg<br>Medulla 18 mmHg | [68] |
| Spectroscopy | LiPc | Pentobarbital | Cortex 15 mmHg<br>Medulla 21 mmHg | Renal artery clamp | Cortex 2 mmHg<br>Medulla 2 mmHg | [69] |
| Spectroscopy | LiPc | Isoflurane | Cortex 50 mmHg | Diabetes induction (alloxan) | Cortex 35 mmHg | [70] |

and medulla decreased during ischemia. One hour after reperfusion, the $pO_2$ values in the untreated group were not fully restored, whereas those groups treated with a urinary trypsin inhibitor were restored to the pre-ischemic values [69]. Of note, both previous studies provided contradictory results regarding oxygen gradient between the cortex and the medulla (Table 2). It could be hypothesized that $pO_2$ values reported in these studies were likely underestimated. Indeed, a more recent study performed under isoflurane anesthesia reported basal cortex $pO_2$ around 50 mmHg [70], emphasizing again the crucial role played by the anesthesia. By monitoring oxygen levels in the kidney cortex of normoglycemic control mice before and after the onset of chemically induced insulinopenic diabetes (alloxan treatment), the authors showed depletion in oxygenation in the renal cortex within the first 3 days after diabetes induction that persisted throughout the 15-day study period [70].

Liver: the reports on oxygenation status observed in the liver are presented in Table 3. The first report of the application of EPR oximetry used a perdeuterated nitroxide encapsulated in liposome after IM or IP administration to mice [71]. Basal $pO_2$ increased from 27 to 63 mmHg by manipulating the oxygen content in air-breathing. In another study, India Ink, administered by IV injection in mice, accumulated in the liver (Kupfer cells) and in the spleen [72]. This study reported a $pO_2$ value of 14 mmHg at the basal state. After intoxication with carbon tetrachloride, the $pO_2$ dropped around 10 mmHg (values extrapolated from the calibration curve provided in this study) [72]. In another study, India Ink and LiPc were used to monitor liver oxygenation in conscious restrained animals before and after anesthesia with pentobarbital, showing the dramatic decrease in liver oxygenation using this anesthetic [73]. Authors also reported differences in $pO_2$ estimates using both probes, India Ink being phagocytized by Kupffer cells while LiPc likely remaining in the extracellular medium [73]. Another publication reported a strategy to optimize the biocompatibility of the oxygen-sensitive materials by decreasing the size of the particles and coating them with suspending or surfactive agents. Small particles of fusinite coated by Arabic gum and intravenously administered to mice accumulated in the liver, whereas the uncoated fusinite was toxic when injected intravenously due to the large size and aggregation of the particles [38]. In a study reporting the effect of nodularin, a cyclic hepatotoxin isolated from the cyanobacterium *Nodularia spumigena*, the $pO_2$ in the liver was measured in vivo in mice using LiPc implanted in the liver as an oxygen sensor [74]. A twofold decrease in hepatic $pO_2$ was reported 2–3 h following nodularin exposure [74].

*Heart* The measurement of oxygen tension in beating heart is a real challenge. Methods have been first developed to evaluate hypoxia in ischemic/reperfusion models of isolated perfused rodent heart [75–78]. A series of studies were performed in mice with Lithium octa-*N*-butoxy-naphthalocyanine to study the role of eNOS on the myocardial $pO_2$ [79]. After thoracotomy, the probe was implanted into the myocardium of the area at risk. In wild-type and $eNOS^{-/-}$ mice, myocardial tissue $pO_2$ dropped from baseline values of 9 and 10 mmHg to 1 and 3 mmHg, respectively at 30 min of coronary ligation. After reperfusion, myocardial $pO_2$ increased markedly, but the $pO_2$ was much lower in $eNOS^{-/-}$ mice (20 mmHg) than in wild-type mice (46 mm Hg) [79]. EPR oximetry has also been used for simultaneous monitoring of stem-cell therapy and in situ oxygenation in a mouse model of acute myocardial

**Table 3** Oxygenation status reported in the liver using EPR oximetry

| Technique | Oxygen sensor | Anesthesia | Oxygenation under Basal/ Physiological conditions | Challenge or Pathology | Oxygenation under challenge or pathological conditions | References |
|---|---|---|---|---|---|---|
| Spectroscopy | Perdeuterated nitroxide in liposomes | Methoxyflurane/Ketamine | 27 mmHg | 85% FiO2 | 63 mmHg | [71] |
| Spectroscopy | India Ink | None | 14 mmHg | $CCl_4$ | 10 mmHg (extrapolated) | [72] |
| Spectroscopy | India Ink | None<br>Pentobarbital | 15 mmHg<br>2 mmHg | | | [73] |
| | LiPc | None<br>Pentobarbital | 23 mmHg<br>10 mmHg | | | |
| Spectroscopy | Fusinite nanoparticles | Ketamine/Xylazine | 19 mmHg | | | [38] |
| Spectroscopy | LiPc | Isoflurane | 20 mmHg | Nodularin | 10 mmHg | [74] |

infarction [80]. The $pO_2$ was significantly higher in infarcted hearts treated with stem cells compared to untreated hearts (Table 4). Another study by the same group evaluated the cardioprotective effect of sulfaphenazole (SPZ), a selective inhibitor of cytochrome P450 2C9 enzyme, in a rat model of acute myocardial infarction [81]. This study established that SPZ attenuated myocardial ischemia/reperfusion injury through overexpression of iNOS, leading to enhancement of nitric oxide bioavailability and tissue oxygenation [81]. The objective of another study was to determine whether trimetazidine (TMZ), given before reperfusion, could attenuate myocardial reperfusion injury [82]. TMZ is an anti-ischemic drug that optimizes cardiac metabolism by reducing fatty acid oxidation through the selective inhibition of mitochondrial 3-ketoacyl CoA thiolase. The results show an oxygen overshoot (hyperoxygenation) during reperfusion in untreated rats. However, in TMZ-treated hearts, the $pO_2$ values remained at near-normal levels (20 mmHg) [82]. A more recent publication reported the effect of exposure to supplemental oxygen cycling on myocardial oxygen tensions in a rat model of acute myocardial infarction [83]. While the breathing of oxygen-enriched gases increased the oxygen tension in healthy hearts, there was no significant improvement of the oxygenation in rat hearts subjected to myocardial infarction.

*Skeletal Muscle* Many studies have monitored the oxygen tension in the skeletal muscle, especially in the gastrocnemius muscle of rodents (mice, rats) [28, 34–36, 38–41, 78, 84–93] (Table 5). The reason for this interest in measuring muscle oxygen tension is more related to the simplicity of the procedure to validate the responsiveness of a new oxygen sensor rather than a focus on muscle (patho)physiology. Indeed, after implantation of an oxygen sensor in a leg muscle of a rodent, it is very easy to induce transient ischemia by interrupting the blood flow with a tourniquet or an elastic. This experimental set-up is the simplest way to verify the capability of the sensor to report on dynamic changes in $pO_2$. Moreover, this method has also been used to monitor the stability of responsiveness over long periods of time by repeating this protocol over weeks or months. As shown in Table 5, a large variety of oxygen sensors has been tested for this purpose, including LiPc, LiNc, LiNc-BuO, several charcoals including approved material for human use, chars, and carbon blacks in commercial India inks or dispersed in pharmaceutical-grade suspending agents. Several probes have been included in biocompatible polymers (Teflon or PDMS) for their use as retrievable inserts or components of implantable resonators. Reported $pO_2$ values from the gastrocnemius muscles varied from 8 to 20 mmHg (with a mean of 15 mmHg) under ketamine/xylazine anesthesia, and from 16 to 40 mmHg (with a mean of 21 mmHg) under isoflurane (Table 5).

*Skin* Reports on oxygenation status observed in the skin are presented in Table 6. Interestingly, the first EPR measurements ever made in a human have been done using the India ink in a pre-existing tattoo [84]. The EPR spectra of India ink (that contains carbon black as an active EPR oxygen sensor) are very sensitive to the partial pressure of oxygen. This first study was done on a volunteer with an extensive tattoo on his lower arm. The black area of the tattoo was positioned under the surface coil used for detection [84]. Spectra were obtained before and after constriction of the blood flow by means of a rubber tourniquet around the arm. The LW of the EPR spectra changed from 4.05 Gauss to 3.4 Gauss. The corresponding values of

**Table 4** Oxygenation status reported in the myocardium using EPR oximetry

| Technique | Oxygen sensor | Anesthesia | Oxygenation under Basal/ Physiological conditions | Challenge or Pathology | Oxygenation under challenge or pathological conditions | References |
|---|---|---|---|---|---|---|
| Spectroscopy | LiNc-BuO | Ketamine/Xylazine | 9 mmHg | Wild-type | | [79] |
| | | | | Coronary ligation | 1 mmHg | |
| | | | | Reperfusion | 46 mmHg | |
| | | | | eNOS-/- | | |
| | | | | Coronary ligation | 3 mmHg | |
| | | | | Reperfusion | 20 mmHg | |
| Spectroscopy | LiNc-BuO | Ketamine/Xylazine | 15 mmHg | Infarcted<br>Infarcted + stem cells | 1.6 mmHg<br>3.5 mmHg | [80] |
| Spectroscopy | LiNc-BuO | Isoflurane | 18 mmHg | Ischemia<br>Untreated/Reperfusion<br>SPZ treated/Reperfusion | 2 mmHg<br>35 mmHg<br>45 mmHg | [81] |
| Spectroscopy | LiNc-BuO | Isoflurane | 20 mmHg | Ischemia<br>Untreated/Reperfusion<br>TMZtreated/Reperfusion | 2 mmHg<br>30 mmHg<br>20 mmHg | [82] |
| Spectroscopy | LiNc-BuO | Isoflurane | 14 mmHg | Carbogen ($95\%O_2/5\%CO_2$)<br>100% $O_2$ | 28 mmHg<br>25 mmHg | [83] |

**Table 5** Oxygenation status reported in the skeletal muscle using EPR oximetry

| Technique | Oxygen sensor | Anesthesia | Oxygenation under Basal/Physiological conditions | Challenge or Pathology | Oxygenation under challenge or pathological conditions | References |
|---|---|---|---|---|---|---|
| Spectroscopy | LiPc | Ketamine | 24 mmHg | Blood flow restriction | 7 mmHg | [28] |
| Spectroscopy | India Ink | Conscious | 14 mmHg | Blood flow restriction | 1 mmHg | [84] |
| Spectroscopy | Fusinite in PDMS | Ketamine/Xylazine | 8 mmHg | Blood flow restriction | 0 mmHg | [41] |
| Spectroscopy | Fusinite in suspending agents | Ketamine/Xylazine | 19 mmHg | Blood flow restriction | 0 mmHg | [38] |
| Spectroscopy | Charcoal | Ketamine/Xykazine | 15 mmHg | Blood flow restriction | 0 mmHg | [34] |
| Spectroscopy | Charcoal coated | Ketamine/Xylazine | 15 mmHg | Blood flow restriction | 0 mmHg | [39] |
| Spectroscopy | Bubinga char coated | Ketamine/Xylazine | 20 mmHg | Blood flow restriction | 0 mmHg | [85] |
| Spectroscopy | LiNc | Isoflurane | 17 mmHg | Blood flow restriction | 1 mmHg | [78] |
| Spectroscopy | Carbon black | Ketamine/Xylazine | 10 mmHg | Blood flow restriction | 0 mmHg | [35] |
| Spectroscopy | Charcoal | Isoflurane | 19 mmHg | Blood flow restriction | 3 mmHg | [86] |
| Spectroscopy | Carbon Black in suspending agents | Ketamine/Xylazine | 14 mmHg | Blood flow restriction | 0 mmHg | [40] |
| Spectroscopy | LiPc | Isoflurane | 29 mmHg (37 °C) | 25 °C<br>37 °C carbogen | 10 mmHg<br>50 mmHg | [87] |
| Spectroscopy | LiPc in Teflon large implants | Isoflurane | 20 mmHg | Blood flow restriction | 3 mmHg | [43] |
| Spectroscopy | LiPc in Teflon micro implants | Ketamine/Xylazine | 18 mmHg | Blood flow restriction | 2 mmHg | [88] |
| Spectroscopy | LiNc-BuO in PDMS implant | Isoflurane | 16 mmHg | Blood flow restriction | 1 mmHg | [89] |
| Spectroscopy | Charcoal | Isoflurane | 20 mmHg | Hyperthyroid mice | 16 mmHg | [90] |
| Spectroscopy | LiPc in Teflon-coated resonator | Isoflurane | 40 mmHg | Carbogen | 100 mmHg | [91] |
| Spectroscopy | LiNc-BuO in PDMS implant | Isoflurane | 20 mmHg | Blood flow restriction | 0 mmHg | [92] |
| Spectroscopy | LiNc-BuO in PDMS implant | Isoflurane | 14–27 mmHg | Blood flow restriction | 0 mmHg | [93] |
| Spectroscopy | Carbo-Rep® | Isoflurane | 22 mmHg | Blood flow restriction | 1 mmHg | [36] |

**Table 6** Oxygenation status reported in the skin using EPR oximetry

| Technique | Oxygen sensor | Anesthesia | Oxygenation under Basal/ Physiological conditions | Challenge or Pathology | Oxygenation under challenge or pathological conditions | References |
|---|---|---|---|---|---|---|
| Spectroscopy | India Ink | Isoflurane (mice)<br>Conscious (human) | 28 mmHg<br>20 mmHg | Blood flow restriction | 0 mmHg<br>0 mmHg | [94] |
| Spectroscopy | Bubinga char | Ketamine/Xylazine | 3 mmHg | Formulations of Benzoyl Nicotinate | 6–11 mmHg | [95] |
| Spectroscopy | LiPc | Isoflurane | db/ + 18 mmHg | 1 day post-wound | 9 mmHg | [96] |
| | | | | 11 days post-wound | 17 mmHg | |
| | | | db/db 18 mmHg | 1 day post-wound | 9 mm Hg | |
| | | | | 11 days post-wound | 11 mmHg | |
| Spectroscopy | LiPc | Isoflurane | db/db 7 weeks-old | Before wound | 22 mmHg | [98] |
| | | | Untreated | 1 day post-wound | 1 mmHg | |
| | | | | 9 days post-wound | 4 mmHg | |
| | | | db/db 7 weeks-old | Before wound | 20 mmHg | |
| | | | LL37-treated | 1 day post-wound | 1 mmHg | |
| | | | | 9 days post-wound | 14 mmHg | |
| | | | db/db 12 weeks-old | Before wound | 12 mmHg | |
| | | | Untreated | 1 day post-wound | 1 mmHg | |
| | | | | 9 days post-wound | 3 mmHg | |
| | | | db/db 12 weeks-old | Before wound | 22 mmHg | |
| | | | LL37-treated | 1 day post-wound | 1 mmHg | |
| | | | | 9 days post-wound | 5 mmHg | |

**Table 6** (continued)

| Technique | Oxygen sensor | Anesthesia | Oxygenation under Basal/ Physiological conditions | Challenge or Pathology | Oxygenation under challenge or pathological conditions | References |
|---|---|---|---|---|---|---|
| Spectroscopy | LiNc-BuO in PDMS "SPOT chip" | None | Surface position | Foot | 8–23 mmHg | [99] |
| | | | Transcutaneous | Arm | 8–22 mmHg | |
| Spectroscopy | LiNc-BuO in PDMS | Not reported | Implanted 67 mmHg | Blood flow restriction | 9 mmHg | [100] |
| | | | Transcutaneous 65 mmHg | Blood flow restriction | 9 mmHg | |

$pO_2$ were unknown because the calibration of the response to $pO_2$ for the material used for the tattoo was not available [84]. Later, a commercial India ink that was calibrated for the oxygen response of the EPR LW was administered in the subcutaneous tissue of rats [94]. It took about two weeks to get stable LW estimates. When equilibrated, the $pO_2$ values reported were around 28 mmHg at the basal level and dropped to about 0 mmHg after blood flow compression. In the same study, a pilot experiment was done on a human healthy volunteer with the ink injected under the skin between the first and second toe of the foot. The $pO_2$ values reported in the human skin were around 20 mmHg at the basal state and dropped near 0 mmHg 10 min after muscle compression. This measurement and the responsiveness to induced transient hypoxia were then stable over months [94]. Other studies have attempted to manipulate skin oxygenation. A study investigated the role of several formulations of benzyl nicotinate, a rubefacient, on skin oxygenation after dermal application [95]. This study reported a basal $pO_2$ before application around 3 mmHg that increased to 6–11 mmHg depending on the formulation used, 30–50 min after the application. It is worth mentioning that the animals were kept under ketamine/xylazine anesthesia in this study [95]. The main interest in measuring $pO_2$ in the skin is because the lack of oxygen is classically described as a major cause of impaired wound healing in diabetic patients. A study assessed the value of EPR oximetry to monitor $pO_2$ in wounds during the healing process in diabetic mouse models [96]. In pedicled flaps, hypoxia was observed early after wounding. While reoxygenation occurred over time in non-diabetic db/+mice, hypoxia was prolonged in the diabetic db/db model (Table 6). This observation was consistent with impaired healing and microangiopathies observed using intravital microscopy. Because oxygen plays a key role in wound healing, treatments to improve hemodynamics and increase wound oxygenation are of particular interest for the treatment of chronic wounds [97]. As an example, one research study investigated the value of EPR oximetry to follow oxygenation in wounds treated by a plasmid-encoding host defense peptide hCAP-18/LL37 [98]. LL37 is primarily an antimicrobial peptide. In addition to its role in innate and adaptive immunology, a proangiogenic activity was described for this peptide. Flaps were created on diabetic mice (7- or 12-week-old db/db mice) presenting different levels of microangiopathy. The hCAP-18/LL37-encoding plasmids were administered in wounds by electroporation. Low-frequency EPR oximetry using LiPc was used to monitor wound oxygenation in flaps during the healing process. A reoxygenation of the flap was observed during the healing process in the 7-week-old db/db treated mice, but not in the untreated mice and the 12-week-old mice. Consistently, the kinetics of excisional wound closure was improved by hCAP-18/LL37 treatment in the 7-week-old db/db but not in the 12-week-old mice [98]. Recently, Tc$pO_2$ EPR oximetry using an oxygen-sensing skin adhesive film, named the superficial perfusion oxygen tension (SPOT) chip, has been developed. The chip is covered with an oxygen-barrier material on one side and secured on the skin by a medical adhesive transfer tape to ensure that only the oxygen that diffuses through the skin surface is measured [99]. Measurements were done on ten healthy human subjects with Tc$pO_2$ values ranging from 7 to 22 mmHg in the forearm skin and 8 to 23 mmHg in the foot. EPR oximetry has also been used and compared to classical Tc$pO_2$ electrode measurement in detecting ischemia of a saphenous

artery-based flap in a rat model [100]. The $pO_2$ values reported in this study were surprisingly high compared to other studies. Of note, the anesthesia used was not reported. $TcpO_2$ electrode measurements were done after stabilization at 44 °C. If EPR oximetry measurements were done at the same temperature, this may contribute to the difference in oxygenation readouts.

*Grafts* dynamic measurements of oxygenation status are of paramount importance in cell therapy and organ transplants. The objective of tissue grafting is to replace diseased or dysfunctional cells with healthy, functioning ones. Formation of an avascular sheath (or 'fibrous encapsulation'), as part of the foreign body reaction, severely limits the applicability of biomedical device implant because of the impaired molecular exchange between the device or the graft and nearby circulation [101]. An example of strategy used to stimulate the neovascularization around implants relies in using co-implanted bone-marrow progenitor cells. It was found that the co-implantation of bone-marrow progenitor cells together with Matrigel in polymeric inserts led to a more pronounced re-oxygenation over time after implantation compared to implant without progenitor cells [101]. EPR oximetry has been applied to monitor the oxygen environment in human ovarian grafts in the early postgrafting period [102]. Ovarian fragments were fixed in the parietal peritoneum of mice. A period of hypoxia was identified before day 5, followed by gradual but significant reoxygenation over the next 5 days, suggesting an active process of graft revascularization [102]. To enhance neovascularization and improve ovarian tissue transplantation, further studies evaluated the capability of adipose tissue-derived stem cells (ASCs) in one or a two-step procedure [103, 104]. Higher rates of oxygenation and vascularization of ovarian tissue, as well as increased follicle survival rates, were detected in the early post-grafting period in the group where the ovarian fragments were grafted in a previously prepared site using ASC-loaded fibrin [104]. Another main application has been in the field of pancreas islets transplantation. Islet transplantation could significantly improve the quality of life and prognosis for selected patients with type 1 diabetes mellitus. An attractive alternative to immunosuppressive drugs is cell immunoisolation by encapsulation in a semipermeable matrix to protect transplanted tissues against immune cells from the recipient as well as against antibodies while preserving the access of oxygen and nutrients to the encapsulated islets. A series of alginate polymers (composed of either high mannuronic or high guluronic content or functionalized with RGD peptide) encapsulating pig islets were administered in the subcutaneous tissue of rats. Among the seven polymers tested, only SLM (sterile lyophilized high mannuronate)-alginates, SLG (sterile lyophilized high guluronate)-alginates, and SLG-RGD alginates showed a $pO_2 > 10$ mm Hg during the 4 weeks of follow-up. SLG alginate showed an increasing $pO_2$ from 10 mm Hg after 1 week up to 25 mm Hg after 4 weeks. $pO_2$ with SLG-RGD varied during the 4 weeks, but the mean $pO_2$ was about 20 mm Hg. In this context, SLM clearly demonstrated constant and much higher oxygenation (around 40 mm Hg) during the entire 4-week follow-up [105]. Consistently, this study showed that SLM-encapsulated pig islets demonstrated no inflammatory/immunologic reactions and islets functioned for up to 60 days without immunosuppression. Another study investigated the potential of bone marrow (BM-MSCs) versus adipose mesenchymal stem cells (AMSCs) to potentiate the oxygenation of

encapsulated islets in a subcutaneous bioartificial pancreas in diabetic rat models [106, 107]. Diabetes and islet encapsulation significantly reduced the oxygenation of a subcutaneous bioartificial pancreas (monocellular device) while AMSCs improved the implant's oxygenation and vascularization. The co-transplantation of islets with BM-MSCs or AMSCs in diabetic rats showed significantly higher graft oxygenation than islets alone (3% and 3.6% $O_2$ for islets + BM-MSCs or AMSCs, respectively, vs. 2.2% for islets alone) [107]. In another context, adipose-derived stromal cells that can potentially improve the local environment of wound bed by angiogenesis and immunomodulation were assessed after implantation. Vessel counts and tissue oxygenation were higher after adipose-derived stromal cell implantation [108]. Dermal oxygenation after thermic lesion increased significantly over time (up to 27 days after implantation) with human acellular collagen matrix plus adipose-derived stromal cells compared to human acellular collagen matrix alone. Consistently significantly higher vessel density was found in the dermis reconstituted with human acellular collagen matrix plus adipose-derived stromal cells in comparison with the dermis in contact with human acellular collagen matrix alone [108]. Recently, EPR imaging studies were proposed to assess oxygen content and heterogeneity using microcapsules encapsulating bone marrow-derived human mesenchymal stem cells doped with LiNc–BuO crystals [109]. The $pO_2$ values reported from these studies on grafts are summarized in Table 7.

## 4 Malignant Tumors

Most malignant solid tumors contain areas with hypoxia resulting in worsened clinical prognosis for cancer patients [110–112]. Tumor hypoxia results from an imbalance between the limited oxygen delivery capacity of the abnormal vasculature and the high oxygen consumption of tumor cells [113, 114]. Tumor hypoxia is a crucial therapeutic issue because it renders solid tumors resistant to radiation therapy. Because of the so-called "oxygen enhancement effect", the radiation dose required to achieve the same biologic effect is about three times higher in hypoxic tissues than in those with normal oxygen levels [115]. Moreover, it has been demonstrated that the hypoxic fraction in solid tumors may: (1) select cells with a more aggressive malignant phenotype, (2) promote uncontrolled angiogenesis and (3) promote metastasis [116]. Considering the negative effect of tumor hypoxia, it is crucial to identify reliable biomarkers that may identify hypoxic tumors to propose hypoxia-adapted treatments. The most qualified technique is undoubtedly oxygen electrodes and more specifically the Eppendorf system that was providing histograms of $pO_2$ distribution within tumors. Reliable measurements of oxygen estimates were provided and this method was the reference method that definitely provides the evidence for the direct relationship between tumor hypoxia and resistance to irradiation in patients. For a review of several dozens of studies, see references [110, 117] and the publication of Peter Vaupel in the same issue of this journal. Over the years, EPR oximetry has been increasingly be used not only to characterize hypoxic tumor phenotypes but also to monitor changes in oxygen status after therapeutic interventions.

**Table 7** Oxygenation status reported in grafts using EPR oximetry

| Technique | Oxygen sensor | Anesthesia | Oxygenation under Basal/Physiological conditions | Challenge or Pathology | Oxygenation under challenge or pathological conditions | References |
|---|---|---|---|---|---|---|
| Spectroscopy | LiNc-BuO | Not reported | Polymeric insert with co-implantation of Matrigel and bone marrow–derived progenitor cells | Matrigel only | | [101] |
| | | | | Day 0 of implantation | 3 mmHg | |
| | | | | Week 4 post-implantation | 10 mmHg | |
| | | | | Matrigel + BM cells | | |
| | | | | Day 0 of implantation | 3 mmHg | |
| | | | | Week 4 post-implantation | 17 mmHg | |
| Spectroscopy | LiPc | Isoflurane | Human ovarian fragments in the parietal peritoneum | 3 days post grafting | 13 mmHg | [102] |
| | | | | 10 days post grafting | 32 mmHg | |
| Spectroscopy | LiPc | Isoflurane | Human ovarian fragments in the parietal peritoneum | Empty fibrin | | [103] |
| | | | | 3 days post-grafting | 6 mmHg | |
| | | | | 14 days post-grafting | 12 mmHg | |
| | | | | ASC-loaded implants | | |
| | | | | 3 days post-grafting | 4 mmHg | |
| | | | | 14 days post-grafting | 16 mmHg | |
| Spectroscopy | LiPc | Isoflurane | Human ovarian fragments in the parietal peritoneum | Prepared site 2 weeks with ASC-loaded implants | | [104] |
| | | | | 3 days post-grafting | 3 mmHg | |
| | | | | 7 days post-grafting | 26 mmHg | |
| Spectroscopy | Charcoal | Isoflurane | Pig pancreas islets in sc tissue | SLG 1 week post-grafting | 10 mmHg | [105] |
| | | | | SLG 4 wee k post-grafting | 25 mmHg | |
| | | | | SLM 1 wee k post-grafting | 40 mmHg | |
| | | | | SLM 4 wee k post-grafting | 40 mmHg | |

**Table 7** (continued)

| Technique | Oxygen sensor | Anesthesia | Oxygenation under Basal/Physiological conditions | Challenge or Pathology | Oxygenation under challenge or pathological conditions | References |
|---|---|---|---|---|---|---|
| Spectroscopy | LiPc | Isoflurane | Pig pancreas islets in sc tissue | 4 weeks post-grafting Non-diabetic | | [106] |
| | | | | MCD | 4.5% $O_2$ | |
| | | | | MCD/islets | 3% $O_2$ | |
| | | | | MCD/islets/AMSC | – | |
| | | | | Diabetic | | |
| | | | | MCD | 3% $O_2$ | |
| | | | | MCD/islets | 2% $O_2$ | |
| | | | | MCD/islets/AMSC | 3% $O_2$ | |
| Spectroscopy | LiPc | Isoflurane | Pig pancreas islets in sc tissue | 4 weeks post-grafting | | [107] |
| | | | | Islets | 2% $O_2$ | |
| | | | | Islets/BM-MSCs | 3% 2 | |
| | | | | MCD/islets/AMSC | 4% $O_2$ | |

In the following paragraphs, we are reviewing and illustrating the main applications of in vivo EPR oximetry in oncology.

*The Early Days of Epr Tumor Oximetry* (Table 8) The first oxygen sensors that were thought convenient for the purpose were nitroxide radicals. Using these soluble chemicals, extensive work has been performed by the group of H.M. Swartz to measure oxygen in the intracellular compartment and within subcellular structures [118–121]. However, while sufficient in vitro, the sensitivity of EPR LWs of common nitroxides to subtle changes in oxygenation is often too limited for in vivo applications in order to provide reliable $pO_2$ values. To increase the sensitivity of the oxygen measurements, H. Halpern suggested the use of nitroxides with a resolved superhyperfine structure (mHCTPO) [15]. Using this probe, he was able to evaluate the oxygenation status in FSa fibrosarcomas. The $pO_2$ measured in tumors varied with the sizes with lower oxygen concentrations in larger tumors [15]. Because relative changes in LW induced by oxygen are much larger for perdeuterated nitroxides, these compounds were also proposed for oximetry purposes in vivo [13]. The $pO_2$ values recorded in tumors were significantly lower than in muscles where the tumors were implanted. From this initial interest for nitroxides as oxygen reporters, came also the problem of the rapid metabolism of these compounds and the loss of paramagnetism. One major factor responsible for the metabolism is the redox status of the cells. The understanding of this contribution of redox status led to the idea of using them as "smart redox sensitive agents" [17–21, 119]. A major step forward in tumor EPR oximetry has been the identification of stable particulate oxygen-sensitive material presenting a much larger sensitivity to variations in oxygenation [28, 122, 123]. First proofs of concepts were done as soon as these materials have been identified [124], demonstrating the ability the technique to measure oxygen with precision around 1 mmHg. Because of their inertness in tissues and their unsurpassed oxygen sensitivity, particulate materials are considered as ideal for long-term longitudinal monitoring of $pO_2$, particularly after therapeutic intervention. In parallel, instrumental developments, and progress in the design of new stable trityl radicals possessing a very narrow LW rendered possible the mapping of oxygen using EPR imaging.

*Tumor Reoxygenation After Irradiation* One of the first applications of EPR oximetry in tumors was the monitoring of early changes in oxygenation after irradiation (Table 9). This hypothesis of reoxygenation is known for several decades in radiotherapy as part of the rule of the 4 *R*s (Repair, Reassortment, Repopulation, Reoxygenation) describing the tumor response to irradiation. Before the application of EPR oximetry in this context, little was known about the dynamics of the oxygenation after irradiation [125]. It has been demonstrated in several tumor strains that one or two days after irradiation, there was an increase of the tumor $pO_2$ as evidenced by EPR oximetry [126–130]. This provides additional motivation for fractionated irradiation and for monitoring $pO_2$ during irradiation treatment to determine the best window of opportunity for irradiating the tumor [131, 132]. The molecular basis of the increase in $pO_2$ has been investigated in several studies. First, the decrease in cell number affected the overall oxygen respiration (killed cells do not breath). Second, it has been demonstrated an increase in endothelial NO synthase with a consequent increase in production of NO

**Table 8** Oxygenation status reported in proof-of-concept studies testing oxygen sensors

| Tumor type | Technique | Oxygen sensor | Anesthesia | Oxygenation under basal conditions | Intervention | Oxygenation after intervention | References |
|---|---|---|---|---|---|---|---|
| FSa fibrosarcoma | Spectroscopy and spectral-spatial imaging | Nitroxide (mHCTPO) | Ketamine/Xylazine | Tumor mass < 0.25 g 50 mmHg<br>Tumor mass > 0.5 g < 10 mmHg | | | [15] |
| MTG-B mammary | Spectroscopy | Nitroxide ($^{15}$ N-PDT) | Awake | 0–3 mmHg | | | [13] |
| MTG-B mammary | Spectroscopy | Fusinite | Awake | Periphery 6 mmHg<br>Center 0.5 mmHg | | | [123] |

**Table 9** Tumor oxygenation status reported after irradiation

| Tumor model | Technique | Oxygen sensor | Anesthesia | Oxygenation under basal conditions | Intervention | Oxygenation after intervention | References |
|---|---|---|---|---|---|---|---|
| MTG-B mammary<br>RIF-1 fibrosarcoma | Spectroscopy | India ink | Awake | MTG-B | 12 h post prradiation | | [126] |
| | | | | 4.3 mmHg | MTG-B | 2 mmHg | |
| | | | | RIF-1 | RIF-1 | 6 mmHg | |
| | | | | 7.3 mmHg | 48 h post irradiation | | |
| | | | | | MTG-B | 3 mmHg | |
| | | | | | RIF-1 | 8 mmHg | |
| MTG-B mammary | Spectroscopy | Fusinite | Awake | 5 mmHg | 6 h post irradiation | 2 mmHg | [127] |
| | | | | | 48 h post irradiation | 7 mmHg | |
| MTG-B mammary<br>RIF-1 fibrosarcoma | Spectroscopy | India ink | Awake | MTG-B | MTG-B | | [128] |
| | | | | 3 mmHg | 6 h post irradiation | 2 mmHg | |
| | | | | | 48 h post irradiation | 3 mmHg | |
| | | | | RIF-1 | RIF-1 | | |
| | | | | 8 mmHg | 24 h post irradiation | 6 mmHg | |
| | | | | | 72 h post irradiation | 8 mmHg | |
| SCC VII | Spectroscopy | LiNc-BuO | Ketamine/Xylazine | 7–13 mmHg | Irradiation | | [129] |
| | | | | | 24 h post irradiation | 13–19 mmHg | |
| C6 and 9L gliomas | Spectroscopy | LiPc | Isoflurane | C6 | Irradiation | 50% increase from baseline | [130] |
| | | | | 9L | Irradiation | No change | |
| | | | | 8–9 mmHg | | | |
| FSaII | Spectroscopy | Charcoal | Isoflurane | FSaII | FSaII | | [130] |
| Fibrosarcoma | | | | 9 mmHg | 2-6 h post irradiation | 22 mmHg | |
| TLT | | | | TLT | TLT | | |

**Table 9** (continued)

| Tumor model | Technique | Oxygen sensor | Anesthesia | Oxygenation under basal conditions | Intervention | Oxygenation after intervention | References |
|---|---|---|---|---|---|---|---|
| Liver tumor | | | | 4 mmHg | 2-6 h post irradiation | 19 mmHg | |
| TLT<br>Liver tumor | Spectroscopy | Charcoal | Isoflurane | 2 mmHg | Control | | [132] |
| | | | | | 1–2 days post starting | 2 mmHg | |
| | | | | | 6 days post starting | 1 mmHg | |
| | | | | | $^{125}$I brachytherapy | | |
| | | | | | 1–2 days post starting | 10 mmHg | |
| | | | | | 6 days post starting | 4 mmHg | |

[133]. The potentiation of the NO-dependent pathway induced a marked increase in tumor blood flow [128, 132, 133]. In addition, the release of NO contributes to the decrease in OCR (oxygen consumption rate) by surviving cells [132]. Overall, these effects contribute to the reoxygenation of tumors after irradiation and appropriate scheduling may be exploited to potentiate the efficacy of radiation therapy [131, 132]. The same reoxygenation effect in parallel to an increase in blood flow has also been demonstrated using EPR oximetry together with dynamic contrast-enhanced MRI [134].

*Effect of Physical Treatments* The importance of timing when combining treatments is also found in photodynamic therapy. Large increases in $pO_2$ have been observed after photodynamic therapy using verteporfin as photosensitizer. A time-window has been identified during which $pO_2$ increased significantly, and tumors were more sensitive to ionizing radiation in this time frame [135, 136]. A more complex response has been observed using photodynamic therapy applied to melanomas. Blood flow (measured by laser-Doppler) and $pO_2$ changes (measured by EPR oximetry) after vascular-targeted photodynamic therapy (V-PDT) or cellular-targeted PDT (C-PDT) using photosensitizer were investigated in melanomas and correlated with long-term tumor responses. V-PDT led to a decrease in $pO_2$ for up to several days, indicating deep and long-lasting hypoxia. However, the decrease in $pO_2$ after C-PDT lasted for only a short period of time and was followed by a large increase in $pO_2$ that lasted for 4–5 days after therapy. Mild and transient hypoxia after C-PDT led to intense $pO_2$ compensatory effects and modest tumor inhibition, while strong and persistent local hypoxia after V-PDT caused tumor growth inhibition [137]. Another study focused on the effect of PDT combined with redaporfin in lung cancer models. PDT causes rapid vascular damage. This effect was expected to produce a dramatic decrease in tumor tissue oxygenation. Surprisingly, EPR oximetric maps of tumors revealed an increase in $pO_2$ values and a decrease in hypoxic fraction early after treatment. Mean $pO_2$ increased from approximately 15 mmHg to approximately 20 mmHg immediately after treatment, then at 48 h returned to the initial level [138]. Among physical-based treatments, it has also been reported that hyperthermia is a strong adjuvant treatment with radiotherapy because it causes tumor reoxygenation. Consistently with the hyperthermia-induced activation of HIF-1 in tumors and its downstream targets, vascular endothelial growth factor (VEGF) and pyruvate dehydrogenase kinase 1 (PDK1), hyperthermia enhances tumor perfusion/vascularization and decreases oxygen consumption [139]. Paradoxically, it was also discovered that mild hypothermia (32 °C) induced a hypometabolism, a lower oxygen consumption by tumor cells leading to an improvement in tumor oxygenation [140]. It was also observed that the electrical stimulation of the sciatic nerve modified the oxygenation status in tumors implanted in the thigh of mice. This increase in tumor oxygenation was the result of a transient increase in tumor blood flow and a decrease in the tumor oxygen consumption that was mediated by a local production of nitric oxide. Those tumor hemodynamic changes resulted in a radiosensitizing effect [141]. The $pO_2$ data from these investigations are summarized in Table 10.

*Pharmacological Challenges:* numerous pharmacological active compounds have been tested to modify tumor hypoxia and render them more prone to respond to anti-cancer treatments.

**Table 10** Tumor oxygenation status reported after "physical" treatment

| Tumor model | Technique | Oxygen sensor | Anesthesia | Oxygenation under basal conditions | Intervention | Oxygenation after intervention | References |
|---|---|---|---|---|---|---|---|
| RIF-1 fibrosarcoma | Spectroscopy | LiPc | Unknown | 3 mmHg | Photodynamic therapy + ALA-PPIX | 9 mmHg | [135] |
| RIF-1 fibrosarcoma | Spectroscopy | LiPc | Isoflurane | 4 mmHg | Photodynamic therapy + veteprofin | 15 mmHg | [136] |
| S91 melanoma | Spectroscopy | LiPc | Medetomidine/ ketamine | 3 mmHg | C-PDT | 10 mmHg | [137] |
| | | | | | V-PDT | 2 mmHg | |
| LLC<br>Lung tumor | Imaging | Nitroxide<br>3-CPO | Isoflurane | 15 mmHg | Photodynamic therapy + redaporfin | 20 mmHg | [138] |
| FSaII fibrosarcoma | Spectroscopy | Charcoal | Isoflurane | 3 mmHg | Mild hypothermia (32 °C)<br>After 90–120 min | 6 mmHg | [140] |
| TLT<br>liver tumor<br>FSaII fibrosarcoma | Spectroscopy | Charcoal | Ketamine/Xylazine | TLT<br>3 mmHg<br>FSaII<br>3 mmHg | 30 min post electric pulses | TLT<br>7 mmHg<br>FSaII<br>8 mmHg | [141] |

A first-class of compounds is composed of agents acting on the vasomotion of vessels (Table 11). In the first demonstration of the ability of EPR oximetry to provide longitudinal measurements of oxygenation after a pharmacological treatment, 34 different vasodilators have been tested in a very large screening [142]. This study included the following classes: angiotensin-converting enzyme inhibitors, calcium antagonists, alpha antagonists, potassium channel openers, beta-blockers, NO donors, and peripheral vasoactive agents. From this list, 24 compounds induced a significant increase in tumor oxygenation after IP injection (Table 11). Several compounds had a profound effect on tumor oxygenation status and were further characterized for their effect on tumor hemodynamics and potential radiosensitizing properties. For example, it was found that xanthinol nicotinate [143], benzylnicotinate [144] and nitrosocaptopril [145] were able to significantly increase tumor growth delay when the irradiation was applied in the time-window of reoxygenation. It was also found that local administration of botulin neurotoxin (BoNT-A) in tumors significantly increases the tumor perfusion and oxygenation, most probably through an inhibition of neurotransmitter release and neurogenic contraction [146]. This study further showed that the opening of the vascular bed induced by BoNT-A offers a way to substantially increase the response of tumors to radiotherapy and chemotherapy [146, 147].

In order to improve the sensitivity of tumors cells to radiation therapy, tumor hypoxia may also be alleviated by decreasing the oxygen consumption rate (OCR) by tumor cells [148]. Mathematical modelling suggested that decreasing the oxygen consumption should be more efficient than increasing oxygen delivery in order to alleviate tumor hypoxia [149]. Several promising strategies targeting the mitochondrial respiration have been identified by EPR oximetry: several compounds led to alleviation of tumor hypoxia and to an increase in sensitivity to irradiation (Table 12). In this context, it was found that insulin is an inhibitor of OCR and has a profound effect on the tumor microenvironment [150, 151]. Following systemic delivery, insulin triggers endogenous nitric oxide (NO) production in tumors through the stimulation of endothelial NO synthase (eNOS) activity. NO regulates mitochondrial respiration by inhibition of the cytochrome c oxidase (complex IV in the mitochondrial respiratory chain). In addition, NO has an intrinsic radiation sensitivity effect [152, 153]. In the same line, NO donors such as isosorbide dinitrate and S-nitrosocaptopril modulated the tumor microenvironment and radiosensitized tumors [154, 155]. NO donors have therefore a triple effect: improvement of tumor hemodynamics, decrease in oxygen consumption and intrinsic radiosentitivity [153]. It was also found that low extracellular pH in tumors promoted the conversion of nitrites into NO, alleviated tumor hypoxia and radiosensitized solid tumors [156]. Anti-inflammatory agents are also modulators of radiosensitivity through inhibition of the OCR. In a pre-clinical study, four non-steroidal anti-inflammatory drugs (NSAIDs) (piroxicam, indomethacin, diclofenac, and NS-398) were tested for their effect on tumor oxygenation [157]. All the NSAIDs tested caused a rapid increase in tumor oxygenation. In addition, when irradiation was applied at the time of maximal reoxygenation, the tumor radiosensitivity was enhanced [157]. This effect of anti-inflammatory agents has been extended to steroid agents such as glucocorticoids (hydrocortisone, dexamethasone, and prednisolone) [158]. These

**Table 11** Tumor oxygenation status reported after pharmacological treatment (vasoactive drugs)

| Tumor model | Technique | Oxygen sensor | Anesthesia | Oxygenation under basal conditions | Intervention | Oxygenation after intervention | References |
|---|---|---|---|---|---|---|---|
| | | | | | Calcium Antagonists | | |
| TLT liver tumor | Spectroscopy | Charcoal | Ketamine/Xylazine | 1–2 mmHg | 30 min post-treatment | | [142] |
| | | | | | Flunarizine | 7 mmHg | |
| | | | | | Bepridil | 5 mmHg | |
| | | | | | Diltiazem | 4 mmHg | |
| | | | | | Nifedipine | 0 mmHg | |
| | | | | | Nicardipine | 4 mmHg | |
| | | | | | Alpha antagonists | | |
| TLT liver tumor | Spectroscopy | Charcoal | Ketamine/Xylazine | 1–2 mmHg | 30 min post-treatment | | [142] |
| | | | | | Naftopidil | 10 mmHg | |
| | | | | | Phentolamine | 1 mmHg | |
| | | | | | Prasozin | 5 mmHg | |
| | | | | | Urapidil | 5 mmHg | |
| | | | | | Phenoxybenzamine | 2 mmHg | |
| | | | | | K channel openers | | |
| TLT liver tumor | Spectroscopy | Charcoal | Ketamine/Xylazine | 1–2 mmHg | 30 min post-treatment | | [142] |
| | | | | | Minoxidil | 6 mmHg | |
| | | | | | Pinacidil | 3 mmHg | |
| | | | | | Diazoxide | 5 mmHg | |
| | | | | | Beta blockers | | |
| TLT liver tumor | Spectroscopy | Charcoal | Ketamine/Xylazine | 1–2 mmHg | 30 min post-treatment | | [142] |
| | | | | | Pindolol | 6 mmHg | |
| | | | | | Alprenolol | 8 mmHg | |

**Table 11** (continued)

| Tumor model | Technique | Oxygen sensor | Anesthesia | Oxygenation under basal conditions | Intervention | Oxygenation after intervention | References |
|---|---|---|---|---|---|---|---|
| | | | | | Atenolol | 5 mmHg | |
| | | | | | Timolol | 6 mmHg | |
| | | | | | Peripheral vasodilators | | |
| TLT liver tumor | Spectroscopy | Charcoal | Ketamine/Xylazine | 1–2 mmHg | 30 min post-treatment | | [142] |
| | | | | | Dihydralazine | 6 mmHg | |
| | | | | | Nicotinamide | 6 mmHg | |
| | | | | | Pentifylline | 2 mmHg | |
| | | | | | Propentofylline | 10 mmHg | |
| | | | | | Pentoxifylline | 8 mmHg | |
| | | | | | Xanthinol nicotinate | 10 mmHg | |
| | | | | | Suloctidil | 6 mmHg | |
| | | | | | Buflomedil | 5 mmHg | |
| | | | | | Bamethan | 1 mmHg | |
| | | | | | Ifenprodil | 1 mmHg | |
| TLT liver tumor | Spectroscopy | Charcoal | Isoflurane | 1 mmHg | 10–30 min post IP treatment | | [143] |
| | | | | | Xanthinol nicotinate | 6 mmHg | |
| RIF-1 fibrosarcoma | Spectroscopy | LiPc | Isoflurane | 5–7 mmHg | 10–30 min post topical treatment | | [144] |
| | | | | | Benzyl nicotinate | 7–17 mmHg | |
| | | | | | ACE inhibitors | | |
| TLT liver tumor | Spectroscopy | Charcoal | Ketamine/Xylazine | 1–2 mmHg | 30 min post-treatment | | [142] |
| | | | | | Captopril | 5 mmHg | |
| | | | | | Enalapril | 3 mmHg | |

**Table 11** (continued)

| Tumor model | Technique | Oxygen sensor | Anesthesia | Oxygenation under basal conditions | Intervention | Oxygenation after intervention | References |
|---|---|---|---|---|---|---|---|
| | | | | | Lisinopril | 6 mmHg | |
| TLT liver tumor | Spectroscopy | Charcoal | Isoflurane | 3 mmHg | 30 min post-treatment | | [145] |
| | | | | | Captopril | 5 mmHg | |
| | | | | | S-nitrosocaptopril | 9 mmHg | |
| | | | | | No donors | | |
| TLT liver tumor | Spectroscopy | Charcoal | Ketamine/Xylazine | 1–2 mmHg | 30 min post-treatment | | [142] |
| | | | | | Isosorbide dinitrate | 8 mmHg | |
| | | | | | Nitroglycerin | 5 mmHg | |
| | | | | | Sodium nitroprusside | 6 mmHg | |
| | | | | | Molsidomine | 6 mmHg | |
| | | | | | Inhibitor of neurotransmetters release | | |
| TLT liver tumor<br>FSaII fibrosarcoma | Spectroscopy | Charcoal | Isoflurane | 1–2 mmHg | 2–4 days post local injection | | [146] |
| | | | | | BoNT-A | | |
| | | | | | TLT | 8 mmHg | |
| | | | | | FSaII | 11 mmHg | |

**Table 12** Tumor oxygenation status reported after pharmacological treatment (OCR inhibitors)

| Tumor model | Technique | Oxygen sensor | Anesthesia | Oxygenation under basal conditions | Intervention | Oxygenation after intervention | References |
|---|---|---|---|---|---|---|---|
| TLT liver tumor | Spectroscopy | Charcoal | Ketamine/Xylazine | TLT | 30 min post slow infusion Insulin | TLT | [150] |
| FSaII fibrosarcoma | | | | 2 mmHg | | 9 mmHg | |
| | | | | FSaII | | FSaII | |
| | | | | 3 mmHg | | 11 mmHg | |
| TLT liver tumor | Spectroscopy | Charcoal | Ketamine/Xylazine | 2 mmHg | 30 min post Isosorbide dinitrate | 7 mmHg | [154] |
| FSaII fibrosarcoma | Spectroscopy | Charcoal | Ketamine/Xylazine | 3 mmHg | 30 min post Isosorbide dinitrate | 13 mmHg | [155] |
| TLT livertumor | Spectroscopy | Charcoal | Ketamine/Xylazine | 3 mmHg | Nitrite | 8 mmHg | [156] |
| TLT liver tumor | Spectroscopy | Charcoal | Isoflurane | | 30 min post NSAIDs | | [157] |
| FSaII fibrosarcoma | | | | TLT | TLT | | |
| | | | | 2 mmHg | Piroxicam | 4 mmHg | |
| | | | | 6 mmHg | Indomethacin | 13 mmHg | |
| | | | | 4 mmHg | Diclofenac | 7 mmHg | |
| | | | | 6 mmHg | NS-398 | 12 mmHg | |
| | | | | FSaII | FSaII | | |
| | | | | 3 mmHg | NS-398 | 6 mmHg | |
| TLT liver tumor | Spectroscopy | Charcoal | Isoflurane | | 30 min post Glucocorticoids | | [158] |
| FSaII fibrosarcoma | | | | TLT | TLT | | |
| | | | | 2 mmHg | Hydrocortisone | 6 mmHg | |
| | | | | FSaII | FSaII | | |
| | | | | 4 mmHg | Hydrocortisone | 7 mmHg | |
| | | | | 3 mmHg | Dexamethasone | 7 mmHg | |
| | | | | 3 mmHg | Prednisolone | 6 mmHg | |

**Table 12** (continued)

| Tumor model | Technique | Oxygen sensor | Anesthesia | Oxygenation under basal conditions | Intervention | Oxygenation after intervention | References |
|---|---|---|---|---|---|---|---|
| TLT liver tumor | Spectroscopy | Charcoal | Isoflurane | | 90 min post $As_2O_3$ | | [159] |
| LLC Lewis lung carcinoma | | | | TLT 3 mmHg | TLT | 40 mmHg | |
| | | | | LLC 3 mmHg | LLC | 11 mmHg | |
| TLT liver tumor | Spectroscopy | Charcoal | Isoflurane | 2 mm Hg | 3 weeks Propyl thiouracile | 11 mmHg | [160] |
| MDA-MB-231 breast cancer | Spectroscopy | Charcoal | Isoflurane | | 30 min post NaHS | | [161] |
| | | | | SiHa | SiHa | 10 mmHg | |
| SiHa | | | | 6 mmHg | | | |
| Cervix carcinoma | | | | MDA-MB-231 | MDA-MB-231 | 15 mmHg | |
| | | | | 6 mmHg | | | |

glucocorticoids caused a rapid increase in tumor oxygenation after administration. OCR was significantly changed while DCE-MRI parameters were unchanged after glucocorticoids treatment. Most importantly, glucocorticoids significantly increased the effectiveness of tumor radiotherapy when irradiation was given at the time of maximal reoxygenation [158]. Another study assessed the effect of arsenic trioxide ($As_2O_3$) on the tumor microenvironment and on the tumor response to irradiation [159]. It was found that arsenic trioxide reduced the tumor hypoxic fraction early after administration. The early increased oxygenation effect was linked to a decrease in tumor cell oxygen consumption rate and, consequently, arsenic trioxide increased the effectiveness of tumor radiotherapy when irradiation was performed in the time window of increased oxygenation [159]. It is worth noting that tumor reoxygenation occurred despite the decrease in tumor blood flow induced by arsenic trioxide. This latter result highlights that the modulation of tumor oxygen consumption is the most critical factor to alleviate tumor hypoxia as previously predicted by theoretical models [149]. Besides drug-induced modulation of the OCR, it is important to realize that physiological status and physiological modulators may also alter the oxygenation status in tumors. In normal tissues, thyroid hormones play a major role in the metabolic activity of cells. In addition, it was demonstrated that thyroid hormones affect the metabolic activity of tumor cells and hence modulate the response to cytotoxic treatments in experimental tumors [160]. Thyroid status significantly modified tumor $pO_2$: hypothyroid mice (through 3 weeks of treatment with propyl thiouracil) presented significantly higher tumor oxygenation compared to euthyroid mice. This was associated with a significant change in tumor radiosensitivity since the regrowth delay was increased in hypothyroid mice compared to euthyroid mice. Another potential physiological modulator of OCR is hydrogen sulfide ($H_2S$), the last gaseous transmitter identified in mammals. One study showed that sodium hydrosulfide (NaHS), a $H_2S$-releasing donor, decreased the OCR in tumor cells, increased tumor oxygenation, and potentiated the response to irradiation [161].

A third class of agents has been tested for the effect on tumor oxygenation: the allosteric hemoglobin modifiers. These compounds may increase the delivery of oxygen by shifting the oxygen equilibrium curve of hemoglobin to the right. Several compounds have been tested by EPR oximetry to monitor their effect on tumor oxygenation status (Table 13). A first study showed that efaproxiral (RSR13) increased the tumor oxygenation in RF-1 fibrosarcomas [162]. In another report, $pO_2$ values found in gliomas before and after efaproxiral treatment were very high [163]. Another study evaluated the oxygenation status during the time course of fractionation irradiation protocols with or without efaproxiral treatment. Compared to untreated tumors, the oxygenation status was at all times of the treatment superior in the efaproxiral-treated group [164]. Another compound, myo-inositol trispyrophosphate (ITPP) has been tested in six different tumor models [165]. It increased the tumor oxygen to different extent depending on tumor type. In addition to its effect on hemoglobin saturation, ITTP did modify the OCR in the different tumor cell lines. The benefit of administering ITTP before irradiation was mixed, depending again on the tumor model [165].

Anti-cancer treatments may also have profound effect on the tumor oxygenation (Table 14). A special focus has been made on anti-angiogenic agents. These

**Table 13** Tumor oxygenation status reported after pharmacological treatment (allosteric hemoglobin modifiers)

| Tumor model | Technique | Oxygen sensor | Anesthesia | Oxygenation under basal conditions | Intervention | Oxygenation after intervention | Reference |
|---|---|---|---|---|---|---|---|
| RIF-1 fibrosarcoma<br>sc implantation | Spectroscopy | LiPc | Isoflurane | 4–6 mmHg | 30 min post efaproxiral | 9–20 mmHg | [162] |
| 9L glioma<br>intracranial | Spectroscopy | LiPc | Isoflurane | 97–110 mmHg | 1–6 days<br>1 h post injection efaproxiral | 139–197 mmHg | [163] |
| RIF-1 fibrosarcoma<br>sc implantation | Spectroscopy | LiPc | Isoflurane | 6 mmHg | 5 days irradiation<br>1 h post efaproxiral | | [164] |
| | | | | | Untreated | 5–14 mmHg | |
| | | | | | Efaproxiral | 16–54 mmHg | |
| 9L glioma<br>Rhabdomyosarcoma<br>NT2<br>Mammary cancer<br>FSaII fibrosarcoma<br>SiHa Cervix carcinoma<br>MDA-MB-231 breast cancer | Spectroscopy | Charcoal | Isoflurane | | ITTP | | [165] |
| | | | | | 2 h-24 h post treatment | | |
| | | | | 9L | 9L | | |
| | | | | 7 mmHg | 15 mmHg | 15 mmHg | |
| | | | | Rhabdomyosarcoma | 9–15 mmHg | 9–15 mmHg | |
| | | | | 5 mmHg | Rhabdomyosarcoma | | |
| | | | | NT2 | NT2 | | |
| | | | | 5 mmHg | 5–10 mmHg | 5–10 mmHg | |
| | | | | FSaII | FSaII | | |
| | | | | 5 mHg | 7 mmHg | 7 mmHg | |
| | | | | SiHa Cervix | SiHa Cervix | | |
| | | | | 5 mmHg | 10 mmHg | 10 mmHg | |
| | | | | MDA-MB-231 | MDA-MB-231 | | |
| | | | | 5 mmHg | 7–8 mmHg | 7–8 mmHg | |

**Table 14** Tumor oxygenation status reported after pharmacological treatment (anti-cancer agents)

| Tumor model | Technique | Oxygen sensor | Anesthesia | Oxygenation under basal conditions | Intervention | Oxygenation after intervention | References |
|---|---|---|---|---|---|---|---|
| FSaII fibrosarcoma | Spectroscopy | Charcoal | Isoflurane | 4 mmHg | 2–3 days post Thalidomide | 15 mmHg | [167] |
| TLT liver tumor | Spectroscopy | Charcoal | Isoflurane | 3 mmHg | 2–3 days post Thalidomide | 17 mmHg | [168] |
| FSaII fibrosarcoma | Spectroscopy | Charcoal | Isoflurane | TLT | 2 days post SU5416 | TLT | [169] |
| TLT liver tumor | | | | 3 mmHg | | 7 mmHg | |
| | | | | FSaII | | FSaII | |
| | | | | 2 mmHg | | 6 mmHg | |
| TLT liver tumor | Spectroscopy | Charcoal | Isoflurane | 2 mmHg | 2 days post Vandenatib | 12 mmHg | [170] |
| FSaII fibrosarcoma | Spectroscopy | Charcoal | Isoflurane | TLT | 4 days post electrotransfer of plasmid pRDD | TLT | [171] |
| TLT liver tumor | | | | 3 mmHg | | 22 mmHg | |
| | | | | FSaII | | FSaII | |
| | | | | 3 mmHg | | 10 mmHg | |
| FSaII fibrosarcoma | Spectroscopy | Charcoal | Isoflurane | 3 mmHg | 2 days post-treatment | | [172] |
| | | | | | Sorafenib | 22 mmHg | |
| | | | | | PD0395201 | 18 mmHg | |
| FSaII fibrosarcoma | Spectroscopy | Charcoal | Isoflurane | TLT | 2 days post-treatment | TLT | [173] |
| | | | | 3 mmHg | Gefitinib | 16 mmHg | |
| TLT liver tumor | | | | FSaII | | FSaII | |
| | | | | 4 mmHg | | 19 mmHg | |
| HNSCC | Spectroscopy | LiPc | Ketamine/Xylazine | UT-SCC-2 | 10 days after start of Cetuximab treatment | UT-SCC-2 | [174] |

**Table 14** (continued)

| Tumor model | Technique | Oxygen sensor | Anesthesia | Oxygenation under basal conditions | Intervention | Oxygenation after intervention | References |
|---|---|---|---|---|---|---|---|
| UT-SCC-2 | | | | 23 mmHg | | 37 mmHg | |
| UT-SCC-14 | | | | UT-SCC-14 | | UT-SCC-14 | |
| | | | | 19 mmHg | | 29 mmHg | |
| TLT liver tumor | Spectroscopy | Charcoal | Isoflurane | 3 mmHg | 24 h post Paclitaxel | 10 mmHg | [175] |
| 9L glioma | Spectroscopy | LiPc | Isoflurane | 6–8 mmHg | Day 18 to 34 during metronomic cyclophosphamide | 30–45 mmHg | [176] |
| SA-1 fibrosarcoma | Specroscopy | Bubinga char | Médétomidine/Ketamine | 5–7 mmHg | 1 h post Vinblastine | 2 mmHg | [177] |
| SA-1 fibrosarcoma | Specroscopy | Bubinga char | Médétomidine/Ketamine | 5–7 mmHg | 2 h post Cisplatin electrochemotherapy | 1 mmHg | [178] |
| NT2 | Spectroscopy | Charcoal | Isoflurane | NT2 | 3 h post Combretastatin A4 | NT2 | [179] |
| MDA-MB-231 | | | | 4 mmHg | | 3 mmHg | |
| Mammary tumors | | | | MDA-MB-231 | | MDA-MB-231 | |
| | | | | 11 mmHg | | 7 mmHg | |
| SCCVII Squamous cell carcinoma | Imaging | Trityl | Isoflurane | Muscle 27 mmHg Tumor 12 mmHg | 10 min post amifostine | Muscle 20 mmHg Tumor 7 mmHg | [180] |
| SCCVII Squamous cell carcinoma | Imaging | Trityl | Isoflurane | 12 mmHg | 30 min post Pyruvate | 9 mmHg | [182] |
| SCCCVII | Imaging | Trityl | Isoflurane | SCCVII | 30 min post Pyruvate | SCCVII | [183] |

**Table 14** (continued)

| Tumor model | Technique | Oxygen sensor | Anesthesia | Oxygenation under basal conditions | Intervention | Oxygenation after intervention | References |
|---|---|---|---|---|---|---|---|
| Squamous cell carcinoma | | | | 18 mmHg | | 11 mmHg | |
| | | | | HT29 | | HT29 | |
| HT29 colon adenocarcinoma | | | | 15 mmHg | | 12 mmHg | |

compounds should decrease the recruitment of vessels in the growing tumors and therefore decrease the oxygen and nutrient supply. However, it has been hypothesized that anti-angiogenic agents, at the early phase of treatment, should "normalize" the tumor vascularization (by pruning the immature vessels), decrease the interstitial fluid pressure, increase the blood flow and improve the tumor oxygenation status [166]. After this transient normalization phase, there should be a decrease in tumor blood flow and oxygenation corresponding to the long-term effect expected from anti-angiogenic agents. This normalization hypothesis provided to EPR oximetry a unique opportunity to analyze the evolution of tumor oxygenation over time because it opened the possibility to identify reoxygenation time-window for the rational application of combined radiation or chemotherapy treatment. The first application of this paradigm was done using thalidomide [167]. It was found a time window of 2–3 days post-treatment with an increase in tumor oxygenation, increase in hemodynamics parameters and decrease in interstitial fluid pressure [167, 168]. When applied in this time window, there was a significant increase in tumor response to radiation therapy or to chemotherapy. Again, this dynamic longitudinal monitoring of tumor $pO_2$ was central to identify the best schedule for combined therapies. For other anti-angiogenic agents such as SU5416 and vandetanib, it turned out that the mechanism of reoxygenation was much more complex [169, 170]. Indeed, for these compounds, no increase in perfusion was observed while profound changes in metabolism and OCR were observed early after initiation of the treatment. As a consequence, these treatments potentiated the effect of radiation therapy but not chemotherapy [169, 170]. The same observation was done using an electrotransfer of a plasmid encoding an antiangiogenic factor, the recombinant disintegrin domain of ADAM-15 (pRDD) [171]. pRDD electrotransfer caused a significant delay in tumor growth. It significantly increased tumor $pO_2$ in the two tumor models tested for at least 4 days. pRDD electrotransfer and radiotherapy were more effective than either treatment alone [171]. Tumor reoxygenation was also observed after the application of other classes of treatment such as MAPKinase inhibitor (Sorafenib and PD0325901) [172]. Reoxygenation was shown after two days of treatments with Sorafenib or PD0325901 in two tumor models, which was further successfully exploited with Sorafenib for improving the radiation response of FSaII tumors. The increase in tumor oxygenation was shown to be the result of two major factors: (i) an increase in blood flow for Sorafenib, that might be linked to its anti-angiogenic effect (vascular normalization), and (ii) a decrease in oxygen consumption for Sorafenib and PD0325901, due to an alteration of the mitochondrial activity [172]. Similar effects were observed after treatments with EGFR inhibitors (gefitinib and cetuximab) [173, 174]. Paclitaxel has been described to radiosensitize tumors. Multiple factors may be at the origin of this radiosensitization including an oxygen effect [175]. EPR oximetry has also been used to identify therapeutic windows during metronomic cyclophosphamide treatment of glioblastomas [176]. Tumor $pO_2$ increased significantly on day 10 and remained at an elevated level until day 33 during 4 weekly treatments with cyclophosphamide. It should be noticed that EPR oximetry was also capable of providing evidence of a decrease in tumor oxygenation associated with the blood flow shut down induced by treatments, such as vinblastine [177], cisplatin [178] and combretastatin [179].

EPR oximetry has also been used to understand tumor oxygenation dynamics that may be beneficial for combined therapies. An illustrative example, EPR oximetric imaging showed that administration of amifostine decreased $pO_2$ in the muscle and also tumor tissues. This finding suggests that lowering the pressure of oxygen in tissues might contribute in part to the radioprotection of amifostine [180]. Another interesting field of the application relies on the stratification of tumors for a treatment using hypoxia-activated prodrugs such as evofosfamide (TH-302). Evofosfamide is a hypoxia-activated prodrug that releases the DNA-damaging bromo-isophosphoramide mustard (Br-IPM) moiety selectively under hypoxic conditions. EPR oximetry showed that the SCCVII tumor had a higher level of hypoxia compared with the HT29 xenograft. Evofosfamide as monotherapy in vivo showed modest effects in SCCVII implants and minimal effects in HT29 xenografts, whereas evofosfamide in combination with ionizing radiation showed significant benefit in both tumor models [181]. Interestingly, compounds that may lead to a transient increase in tumor hypoxia may be beneficial for evofosfamide therapy. In this context, it was found that the injection of pyruvate (the compound used in the evaluation of the glycolytic activity of tumors using hyperpolarization) may lead to transient hypoxia [182]. This observation led to the rationale for combining pyruvate together with evofosfamide in order to increase the cytotoxicity in tumors [183, 184].

*Effect of Oxygen/Carbogen Breathing* Another simple and efficient technique to manipulate oxygenation status and alleviate tumor hypoxia is to let the subject breath a gas enriched in oxygen, for example, 100% oxygen or carbogen ($95\%O_2/5\%CO_2$ or $98\%O_2/2\%CO_2$) (Table 15 for illustrative examples). It should be emphasized that many studies presented earlier with pharmacological manipulation of tumor oxygenation have included oxygen or carbogen breathing as "positive" controls [142]. The hyperoxygenation has been successfully applied to increase tumor oxygenation in gliomas [] [185–189], rhabdomyosarcoma and breast tumors [190]. As described in the next paragraph, several studies correlated the increase in tumor oxygenation with radiation response. While oxygen or carbogen breathing is often more efficient in the magnitude of effect on tumor oxygenation, it should be noticed that some pharmacological approaches have led to a better response to irradiation compared to oxygen/carbogen challenge. This is due in part because pharmacological agents may act by several mechanisms including intrinsic radiosensititizing properties [153].

*Predictor of Response to Radiation Therapy* The ability of EPR oximetry to provide quantitative estimates of tumor $pO_2$ may have a profound effect in the management of patients for radiation therapy. Most studies described earlier in this paper about pharmacological interventions indeed correlated the better response to irradiation to positive changes in tumor oxygenation [17]. We will not include them again in the present discussion. Here, we highlight some additional studies that assess the $pO_2$ estimates as a prognostic indicator of differential response to irradiation (Table 16) [191]. An early report associated tumor $pO_2$ and response to radioimmunotherapy [192]. In non-small cell lung carcinomas, the greatest growth delay in response to radioimmunotherapy was observed for tumors with the highest initial $pO_2$, and the fastest-growing tumors had the lowest initial $pO_2$. In another study, EPR oxygen imaging was investigated for its power to predict the success of tumor control according to tumor oxygenation level and radiation dose [193]. In this study,

**Table 15** Tumor oxygenation status reported after oxygen/carbogen challenge

| Tumor model | Technique | Oxygen sensor | Anesthesia | Oxygenation under basal conditions | Intervention | Oxygenation after intervention | References |
|---|---|---|---|---|---|---|---|
| TLT liver tumor | Spectroscopy | Charcoal | Ketamine/Xylazine | 1–2 mmHg | 30 min | | [142] |
| | | | | | Post-oxygen | 15 mmHg | |
| | | | | | Post-carbogen | 24 mmHg | |
| 9L glioma | Spectroscopy | LiPc | Isoflurane | 9L | 60 min post carbogen | 9L | [185] |
| C6 glioma | | | | 31 mmHg | | 100 mmHg | |
| | | | | C6 | | C6 | |
| | | | | 14 mmHg | | 40 mmHg | |
| F98 glioma | Spectroscopy | LiPc | Isoflurane | 8–16 mmHg | 60 min post carbogen | 15–50 mmHg | [186] |
| F98 glioma | Spectroscopy | LiPc implantable resonator | Isoflurane | 7–38 mmHg | 30 min post carbogen | 13–111 mmHg | [187] |
| U251 glioma | Spectroscopy | LiPc implantable resonator | Isoflurane | 22 mmHg | $pO_2$ max after carbogen | 54 mmHg | [188] |
| 9L glioma | Spectroscopy | Charcoal | Isoflurane | 9L glioma | 30 min post carbogen | 9L glioma | [189] |
| Rhabdomyosarcoma | | | | 8 mmHg | | 11 mmHg | |
| | | | | Rhabdomyosarcoma | | Rhabdomyosarcoma | |
| | | | | 7 mmHg | | 9 mmHg | |
| MDA-MB-231 breast tumor | Spectroscopy | LiNc-BuO | Isoflurane | 3–4 mmHg | 20 min post 100% $O_2$ | 4–5 mmHg | [190] |
| | | | | | 24 h post PTX + 100% $O_2$ | 8 mmHg | |

**Table 16** Tumor oxygenation status as a prognostic marker to tumor response

| Tumor model | Technique | Oxygen sensor | Anesthesia | Oxygenation Under basal conditions | Treatment | Predictivity | References |
|---|---|---|---|---|---|---|---|
| CALU-3 | Spectroscopy | Charcoal | Isoflurane | Mean: 16 mmHg | $^{131}$I antibody against EGP-1 | Poor responders | [192] |
| Non-small cell lung carcinoma | | | | | | 2–14 mmHg | |
| | | | | | | Best responders | |
| | | | | | | 5–39 mmHg | |
| FSa fibrosarcoma | Imaging | Trityl | Isoflurane | Use of HF3, HF6 and HF10 as marker of hypoxia | X-Rays | HF10 is the best prognostic marker of tumor response | [193] |
| FSa fibrosarcoma MCa4 carcinoma | Imaging | Trityl | Isoflurane | FSa | X-Rays | FSA | [194] |
| | | | | HF10: 0–27% | | Controlled tumors | |
| | | | | MCa4 | | HF10: 11% | |
| | | | | HF10: 0–64 | | Failure | |
| | | | | | | HF10: 7%<br>MCa4<br>Controlled tumors<br>HF10: 19%<br>Failure<br>HF10: 35% | |
| U87 and U251 glioma | Imaging | Trityl | Isoflurane | U87 median 30 mmHg | X-Rays | Radio response ratio | [196] |
| | | | | U251 median 18 mmHg | | U87: 1.45<br>U251:1.29 | |
| RIF-1 fibrosarcoma | Spectroscopy | LiPc | Isoflurane | 7 mmHg | X-Rays<br>2× 10 Gy | | [197] |
| | | | | | 2nd dose oxygenated | 16 mmHg | |

**Table 16** (continued)

| Tumor model | Technique | Oxygen sensor | Anesthesia | Oxygenation Under basal conditions | Treatment | Predictivity | References |
|---|---|---|---|---|---|---|---|
| | | | | | Doubling time | 16.8 days | |
| | | | | | 2nd dose hypoxic | 6 mmHg | |
| | | | | | Doubling time | 8.6 days | |

it was found that HF10 (the % of voxels with $pO_2$ lower than 10 mmHg) provides the most significant $pO_2$ statistic to distinguish cured from failed tumors. In another study, HF10 obtained from EPR images showed statistically significant differences between tumors that were controlled by the TCD50 and those that were not controlled for FSa and MCa4 models [194]. Kaplan–Meier analysis of both types of tumors showed that approximately 90% of mildly hypoxic tumors were controlled (HF10% < 10%), and only 37% (FSA) and 23% (MCa4) tumors were controlled if hypoxic. Another EPR oximetry study suggested that treatment delivering a radiation boost to hypoxic volumes led to better tumor control compared to boosts to well-oxygenated volumes [195]. The influence on basal $pO_2$ at the time of irradiation was also investigated in glioma models [196]. The more hypoxic U251 model had a significantly lower response to irradiation compared to the better oxygenated U87 model [196]. EPR oximetry has also been used to investigate the role of dynamics in reoxygenation on tumor response in fractionated radiotherapy. It was observed that tumors remaining hypoxic at the time of the second irradiation presented a shorter doubling time than the ones irradiated after significant reoxygenation [197].

*Comparison with Other Modalities* at the early time of its applications, EPR oximetry has been compared with other techniques able to quantitatively report real oxygenation status. This was achieved during several pharmacological or oxygen-breathing challenges where EPR was compared with techniques such as quenching of fluorescence probes (OxyLite®) [150, 155, 198, 199] or Eppendorf polarographic electrodes [23]. Studies comparing EPR oximetry with fluorescence quenching probes were applied during pharmacological challenges, demonstrating a real-time comparable evolution of oxygenation status using both techniques [150, 155]. Other studies comparing OxyLite with EPR oximetry measurements reported that $pO_2$ values reported by the two methods were similar, that both methods can record a baseline and rapid changes in $pO_2$ and changes in $pO_2$ induced by increasing $FiO_2$ with carbogen were similar by the two methods [198]. In another study applied to tumors, striking differences have been observed between the EPR and OxyLite readings. The differences were attributed to the volume of tissue under examination and the effect of needle invasion at the site of measurement [199]. EPR oximetric images were also compared to OxyLite measurements [23]. This study concluded that the correlation was good both in terms of spatial distribution pattern and $pO_2$ magnitude. EPR oximetry data with implanted LiPc as the oxygen-sensitive paramagnetic material has been also compared with Eppendorf measurements in the brain cortex. From these studies, the main conclusion was that the average $pO_2$ measured by the two methods was similar but EPR reported a significantly higher average $pO_2$, and the Eppendorf reported a larger range of values [200].

Besides the comparison with invasive techniques, EPR oximetry has also been compared to $T_2$* BOLD (Blood Oxygen Level Dependent) MRI. The contrast in MRI depends on the variation in the ratio deoxyhemoglobin (paramagnetic)/oxyhemoglobin (diamagnetic) and is sensitive to "inflow" effect and blood volume fraction [201, 202]. From these comparison studies, the following conclusions can be drawn. When the evolution of oxygenation is positively correlated to an increase in perfusion, $R_2$* ($1/T_2$*) changes were consistent with $pO_2$ increases [203–205]. However, no change in $R_2$* have been observed when the change in oxygenation

status was induced by modulators of oxygen consumption [206] or hemoglobin allosteric effectors [162]. EPR oximetry has also been used to evaluate the value of $T_1$-NMR-based methods in which the contrast is induced by the changes in $T_1$ induced by paramagnetic oxygen that should be considered as a $T_1$ endogenous contrast agent [189, 207, 208]. Another method that has been proposed to image tumor hypoxia is PET with radiolabeled nitroimidazoles. These compounds enter cells and undergo a succession of reduction steps. In the presence of oxygen, the first step is reversible; consequently, the reduced nitroimidazole is immediately re-oxidized and washed out from tissues. Under hypoxic conditions, the re-oxidation is slow, and that allows further reduction to occur. The compound can thereby bind covalently to intracellular macromolecules and be retained inside cells. Due to the need for enzyme system to reduce and bind nitroimidazole, these tracers accumulate selectively only in viable hypoxic cells. However, it is an indirect method that cannot provide an absolute value of $pO_2$. As little information was available on the critical $pO_2$ values under which nitroimidazoles are accumulating in hypoxic tissues, EPR oximetry has been applied to address this issue. It was found that [$^{18}$F]-EF5 [209] and [$^{18}$F]-FAZA [210] accumulated in tumors under 10 mmHg. The combination of EPR oximetry with [$^{18}$F]-FAZA-PET also allowed identifying threshold values in tracer accumulation to guide hypoxia-driven interventions such as the need for carbogen breathing, dose-escalation or combination with the hypoxia-sensitizer nimorazole [211, 212]. Another important application of EPR oximetry has been its comparison with metabolic markers coming from $^1$H-MRS with focus on lactate [213] or from glycolytic fluxes as measured by hyperpolarization of $^{13}$C-pyruvate [214]. This exquisite combination allowed to identify tumors with Warburg phenotype as well as biomarkers of response to treatments targeting hypoxia and glycolysis [215–218].

*Characterization of Cycling Hypoxia* Another area that has received much less attention so far is tumor acute hypoxia, also name fluctuating hypoxia or cycling hypoxia. Acute hypoxia (transient cycles of hypoxia-reoxygenation) is due to transient interruption of flux of red blood cells. This phenomenon is known to occur in solid tumors and may be a poorly appreciated therapeutic problem as it can be associated with resistance to radiation therapy, impaired delivery of chemotherapeutic agents, or metastasis development [219, 220]. Historically, acute changes over time have been characterized using intravital microscopy and histologically based 'mismatch' techniques. The first non-invasive technology that has been used to detect tumor cycling hypoxia has been MRI using the BOLD contrast [221, 222]. However, the method was qualitative and did not inform on real $pO_2$ fluctuations over time. This prompted the development of rapid quantitative techniques such as $^{19}$F-MRI [223] and EPR oximetry [224–226] to map areas of fluctuating hypoxia. The first proof-of-concept demonstrating the ability of EPR oxygen imaging to map spontaneous fluctuations of tumor oxygenation was applied on SCCVII squamous cell carcinomas and HT29 colon adenocarcinomas [224]. Cycles of hypoxia and reoxygenation have been observed with a magnitude of changes that were dependent on tumor size and tumor type (Table 17). Another study assessed the effect of angiogenesis inhibitors on spontaneous oxygen fluctuations [225]. As pointed out earlier, angiogenesis inhibitors have been shown to transiently normalize the tumor vasculatures

**Table 17** Tumor oxygenation status: cycling hypoxia

| Tumor model | Technique | Oxygen sensor | Anesthesia | Oxygenation under basal conditions | Treatment | Cycles observed | References |
|---|---|---|---|---|---|---|---|
| SCCVII<br>Squamous cell carcinoma | Imaging | Trityl | Isoflurane | SCCVII<br>0–35 mmHg<br>HT29<br>0–20 mmHg | None | SCCVII<br>Small tumor<br>SD $pO_2 = 4.6$ mmHg<br>Large tumor<br>SD $pO_2 = 6.4$ mmHg<br>HT29<br>Small tumor<br>SD $pO_2 = 3.7$ mmHg<br>Large tumor<br>SD $pO_2 = 4.1$ mmHg | [224] |
| HT29 colon adenocarcinoma | | | | | | | |
| SCCVII<br>Squamous cell carcinoma | Imaging | Trityl | Isoflurane | Before treatment<br>0–20 mmHg | Sunitinib | After sunitinib<br>8–40 mmHg<br>Untreated<br>SD $pO_2 = 6$ mmHg<br>Sunitinib<br>SD $pO_2 = 4$ mmHg | [225] |
| MCA4 mammary tumor | Imaging | Trityl | Isoflurane | | none | Fluctuations<br>0–25 mmHg | [226] |

and enhance tumor response to treatments. However, the effect of antiangiogenic therapy on cycling tumor hypoxia remained unknown. It was found that early treatment with sunitinib delayed the progression of tumor hypoxia and suppressed the extent of temporal fluctuations in tumor $pO_2$ during the vascular normalization window, resulting in the decrease of cycling tumor hypoxia [225]. Simulated and experimental studies also showed that principal component analysis filtering increased the signal-to-noise ratio for small numbers of sub-volumes with changing $pO_2$, enabling an increase in temporal resolution with minimal deterioration in spatial resolution [226].

HUMAN DATA: The publication entitled "India Ink: A Potential Clinically Applicable EPR Oximetry Probe" has been published in 1994 [227]. With hindsight, it seems that the application of EPR oximetry in humans and in patients has been extremely slow. This could be surprising given that EPR oximetry almost uniquely can make repeated and accurate measurements of $pO_2$ in tissues. Such measurements can provide clinicians with information that can impact directly on diagnosis and therapy, especially for oncology [228]. The challenges for achieving full implementation included considerable effort in instrumental developments for adapting the spectrometer for safe and comfortable measurements in human subjects and for achieving sufficient sensitivity for measurements at the sites of the pathophysiological processes that are being measured [229, 230]. The clinical applicability also required the development of biocompatible probes, the compliance with regulatory constraints to be used in humans, and safety assessments in clinical studies [38–47, 230–233]. A limited number of $pO_2$ results has been published so far in the literature using biocompatible inks [228, 230, 234, 235]. Most studies have been performed in superficial tumors using a 1 GHz spectrometer (ClinEPR). Measurements were generally done first on patients breathing normal air, and then breathing 100% oxygen. The $pO_2$ values recorded in these superficial tumors (lymphoma, melanoma, sarcoma) were mostly under 10 mmHg at the basal state. During the period of 100% oxygen breathing, the results were quite variable between patients, some tumors remaining anoxic, other presenting a small increase in oxygenation, and a small proportion showing a very large increase in oxygenation [228, 230]. Another study on nine patients assessed the feasibility and reproducibility of EPR oximetry to measure temporal changes in the oxygenation of normal breast tissue during and after radiation therapy [235]. The rationale for this study was to explore the potential role of oxygenation on skin fibrosis that often develops radiation-induced toxicities after radiation therapy. Patients were measured every week during radiation therapy (RT) and every 2–3 months follow-ups using EPR oximetry. For all patients, an average of 8–9 measurements was taken for a total of 73 measurements across patients. During their RT, the average baseline $pO_2$ value across patients was $7 \pm 3$ mmHg. When hyperoxygenation was applied, there was a statistically significant rise of 27 mmHg. Following completion of RT, the baseline and hyperoxygenation values were $11 \pm 2$ mmHg and $25 \pm 6$ mmHg, respectively. For both baseline and hyperoxygenation, no significant difference was observed between measurements taken during and after radiation therapy. These results indicate that EPR oximetry may be used in clinical trials to investigate oxygen levels and their response to potentially hyperoxygenation interventions, which could be very useful in determining the clinical

efficacy of radiosensitization and the mechanism of fibrogenesis [235]. Another clinical trial is presently ongoing in cancer patients using LiNc-BuO embedded in PDMS implant (OxyChip). The results of the safety part made in 24 patients has been recently published [233]. Preliminary results about oxygen measurements and response to oxygen-breathing challenge have been announced in meetings. But at the day of submission of this paper, no report on $pO_2$ values has been published in the scientific literature from this clinical trial.

## 5 Conclusion

EPR oximetry is a continuously evolving technology that allows measurements of $pO_2$ values with high sensitivity and reproducibility. The main virtue of EPR oximetry using particulate materials is the unique ability to repeat oxygen measurements from the same site over long periods of time. The longitudinal monitoring of oxygenation status is clearly unique for monitoring physiopathological processes and the effect of treatments aimed at manipulating tissue oxygenation. This review has presented numerous examples of applications in different tissues and in oncology. The ambition of this paper was not to be complete (please forgive me for those reports that were not cited), but to illustrate the unique data provided by the technology in a large diversity of normal tissues, pathological tissues, and tumors. It is, of course, impossible to summarize the richness of all the cited papers in single numbers and interventions as provided in the Tables. However, we expect that this database will be a useful $pO_2$ database as a means of comparison with future data. More importantly, this paper should be considered as an enticement to discover or re-discover the nice contributions made by the researchers of the EPR community in oximetry.

**Acknowledgements** I would like to express my gratitude to my dream team (docs and post-docs) who have produced, during these last 25 years, an incredible amount of excellent data in many challenging experimental models. I would like also to express my gratitude to Hal Swartz, my post-doc mentor in 1993-1994. Since then, I have appreciated his continuous support from him and Ann Flood, his wife, for my scientific activities. This article in this special issue for his 85th birthday is dedicated to him.

**Funding** This research has been supported by the Foundation against Cancer (2016–087) and the Fonds National de la Recherche Scientifique FNRS (7652717F and J008220F).

**Declaration**

**Conflict of interest** None.

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