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Development of the Implantable Resonator System for Clinical EPR Oximetry

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Abstract

Hypoxic tumors are more resistant to radiotherapy and chemotherapy, which decreases the efficacy of these common forms of treatment. We have been developing implantable paramagnetic particulates to measure oxygen in vivo using electron paramagnetic resonance. Once implanted, oxygen can be measured repeatedly and non-invasively in superficial tissues (<3 cm deep), using an electron paramagnetic resonance spectrometer and an external surface-loop resonator. To significantly extend the clinical applications of electron paramagnetic resonance oximetry, we developed an implantable resonator system to obtain measurements at deeper sites. This system has been used to successfully obtain oxygen measurements in animal studies for several years. We report here on recent developments needed to meet the regulatory requirements to make this technology available for clinical use. radio frequency heating is discussed and magnetic resonance compatibility testing of the device has been carried out by a Good Laboratory Practice-certified laboratory. The geometry of the implantable resonator has been modified to meet our focused goal of verifying safety and efficacy for the proposed use of intracranial measurements and also for future use in tissue sites other than the brain. We have encapsulated the device within a smooth cylindrical-shaped silicone elastomer to prevent tissues from adhering to the device and to limit perturbation of tissue during implantation and removal. We have modified the configuration for simultaneously measuring oxygen at multiple sites by developing a linear array of oxygen sensing probes, which each provide independent measurements. If positive results are obtained in additional studies which evaluate bio-compatibility and chemical characterization, we believe the implantable resonator will be at a suitable stage for initial testing in human subjects.

Keywords

Implantable resonator; pO₂; Oxygen; L-band EPR; Clinical applications

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Compliance with Ethical Standards

Conflict of Interest: The authors declare that they have no competing interests.

Introduction

Solid tumors characteristically grow chaotically and often form disordered networks of blood vessels via neoangiogenesis. The resulting structure can impede oxygen delivery through suboptimal perfusion and increased intratumoral pressure. This in conjunction with unconstrained cellular growth, leads to hypoxia in the tumors. The efficacy of radiotherapy is greatly by hypoxia, which has been known qualitatively since at least 1909. Over 60 years ago, Gray et al. demonstrated that the relative radiosensitivity of hypoxic tissues increases by up to a factor of three when irradiated in oxygenated conditions [1]. Hypoxia can also decrease the effectiveness of chemotherapy and immunotherapy [2, 3]. Hypoxia also induces many molecular signaling factors that lead to increased metastasis and local aggressiveness of solid tumors [4].

Despite evidence regarding the importance of oxygen to impact the efficacy of radiation therapy, this relationship has not been thoroughly investigated and exploited in clinical practice. This is because it is important to be able to monitor oxygen in tumors throughout the course of treatment, and there has not to date been a reliable and repeatable method to directly measure oxygen in tissues, i.e., that would also be clinically feasible to implement throughout routine patient care.

Techniques such as fluoro-misonidazole positron emission tomography [5], magnetic resonance imaging (MRI) techniques such as blood-oxygen-level dependent, perfusion, and diffusion imaging, and magnetic resonance (MR) spectroscopy can provide information that can often be related to tissue oxygen if additional information is available, but in themselves do not provide quantitative information on oxygen levels. Eppendorf oxygen-sensitive electrodes were once considered the “gold-standard” of clinical oxygen measurements in tissues, but they were technically difficult to use and had limited use in the same site due to the potential physical damage to tissues associated with its use [6]. Consequently, they are no longer commercially available or supported [7]. Phosphorescent probes have not been developed for clinical use, possibly because they would need to be used in a manner similar to that of oxygen electrodes [8].

Electron paramagnetic resonance (EPR) oximetry using particulate paramagnetic materials provides a potential clinically feasible solution. It is a reliable, repeatable method that directly measures the level of oxygen in tissues [9], and has already been translated into clinical use. The oxygen-sensitive paramagnetic material must be invasively administered, but measurements can then be made and repeated non-invasively [10].

One oxygen-sensitive material we are currently studying for use with EPR oximetry is a paramagnetic microcrystal composed of lithium octa-n-butoxynaphthalocyanine (LiNc–BuO) contained within an oxygen-permeable silicone polymer [11]. This has shown promise in sensitivity and function during oxygen measurements in a currently ongoing Food and Drug Administration (FDA) Phase 1 Trial of Safety/Efficacy [12]. However, due to the non-resonant absorption of 1.2 GHz microwave energy in tissue, direct measurements with surface loop resonators are limited to superficial tissues (approximately 3 cm from the surface of the body).

To make measurements at deeper sites, we have designed and tested an implantable resonator (IR) system in pre-clinical studies. The IR system consists of an implanted device containing multiple oxygen sensing regions, an external surface loop resonator, and EPR spectrometer. The basic wire-structure of the implanted device (shown in Fig. 1) consists of four sensor loops containing oxygen sensitive paramagnetic material (LiNc–BuO contained within an oxygen-permeable silicone elastomer) located at the distal tip of a transmission line that is implanted into deeper tumor tissues. At its proximal end, the transmission line terminates with a coupling loop that is designed to be implanted near the body surface, e.g., within the subcutaneous region of the skin. The coupling loop enables an external surface loop resonator [13], connected to an EPR spectrometer, to transmit radio frequency (RF) energy to the implanted device suitable for EPR detection from the oxygen sensitive paramagnetic materials within the sensor loops embedded into the tissues of interest.

Studies in animals have demonstrated the reliability and repeatability of measurements obtained from IRs in vivo [14–20]. These studies have demonstrated feasibility and have also led to improvements in the utility of the IR system. The lessons learned from these studies have facilitated the transformation of the IR system into an investigational medical device, which we plan to test in a first-in-human Phase 1 clinical trial.

In addition, we were guided by the clinical principles delineated by Swartz et al. when evaluating the major challenges associated with translating EPR technologies into clinical applications, i.e., the importance of addressing the practical and clinical needs for instrumentation in clinical practice, for successfully administering the material/ device in patients while fitting into usual clinical flow, and for meeting the regulatory demands for medical devices [9, 21]. To that end, we focused on aspects pertaining to the safety of the device, its efficacy, and practical features that would lead to its acceptability in clinical practice.

In particular, the design goals we identified from pre-clinical and in vitro testing to further improve the IR for human use were to: (i) keep the paramagnetic material firmly seated in the sensor loops, (ii) maintain the desired shape of the implant in vivo and avoid any possibility of breakage, (iii) increase the overall manufacturability and reproducibility of the physical device, and (iv) incorporate a design deemed suitable by clinicians for human subjects, e.g., easy to implant and causing the least perturbation of tissues.

In this paper, we provide a technical delineation of crucial aspects and progress that has contributed to the final design of the IR system to use in initial human studies. We also provide a delineation of human interface needs, as we see necessary, to translate the device from its preclinical use to clinical applications, including meeting regulatory requirements.

Delineation of Needs for Clinical Use

Our initial preclinical and in vitro studies have demonstrated the validity of oxygen measurements obtained with the IR system. We are now seeking to translate our developments performed in bench testing and animal models into use in human subjects.

The IR system must be shown to have appropriate safety, effectiveness, and clinical acceptance for translation into the clinical setting.

In order for the IR system to have an impact for patients in the future, physicians need to feel confident of the appropriateness and effectiveness of the device. Implantation and measurement procedures must be incorporated in a way that is compatible with usual care of these cancer patients. This extends from evaluating the placement and any biological adverse responses to the device during initial implantation to evaluating its ease of use and its effectiveness during EPR measurements and, if deemed necessary, to removing it intact. We have taken these aspects into consideration in developing the device, especially by involving surgeons and radiation oncologists in the design, testing, and evaluation of improvements of the device at each stage of development. We anticipate that by developing it in collaboration with those who will be using the IR system in human subjects we will make the system more likely to be accepted into the clinical-setting.

Delineation of Technical Aspects and Progress

Transmission line

Various models of the implanted device, shown in Fig. 2, have been tested in animal tissues such as the brain, heart, and skeletal muscle [14–20]. The initial models shown in Fig. 2, Panels a–c consisted of a copper wire transmission line. Copper was chosen initially because of its conductive metallic properties. However, when the copper devices were tested in actively moving tissues in animal models, such as in the flank and neck of a pig, breaks were observed along the length of the transmission line, likely due to work/strain hardening of the metal.

Subsequently, using similar preclinical studies, transmission lines made from nickel-cobalt-chromium-molybdenum alloy (MP35N, acquired from California Wire Co.) were tested. The wire is insulated with polyimide enamel and is 0.004 in diameter before twisted into a transmission line. We found that these wires did not break in vivo (Fig. 2d). In a recent study in rats, six implants with MP35N transmission lines remained intact during a 6-week study [17].

Since then, we conducted tensile testing of the MP35N transmission line according to the American Society of the International Association for Testing and Materials (ASTM) Standard Specification for Metallic Implantable Strands and Cables (F2180-02(2015)). We found that the twisted MP35N wire surpassed the required minimum breaking forces identified in these standards (25.12 and 12.85 N, respectively) [17]. MP35N is also used in other medical devices such as pacing leads, catheters, and orthopedic cables [22], which is appealing with respect to the prospect of the FDA approving the IR system as a medical device. We also plan to vary the length of the transmission line (up to 15 cm) to suit a variety of tumor depths and anatomies.

While the transmission line made from non-magnetic MP35N did not break in vivo, the specific resistivity of the MP35N alloy we are using is 103.3 $\mu\Omega$ -cm, compared to 1.68 $\mu\Omega$ -cm of previously used copper. However, signal sensitivity has not been an issue as we have

seen that oxygen can still be measured reliably. We have also further evaluated issues of RF heating by conducting FDA approved Finite Element Method ANSYS HFSS simulations. The specific absorption rate (SAR) of human skin, fat, bone, cerebrospinal fluid, and brain layers were modeled and imported into simulation. A local increase in temperature was calculated according to IEEE standards and the mentioned tissue layers experienced SAR levels of 1.263, 0.049, 0.002, 0.039, and 0.001 W/kg, respectively.

Patients with IR system implants may also require MR imaging, so we needed to ensure the implant is MRI compatible. Accordingly, MRI compatibility testing has been carried out in accordance with ASTM standards F2052, F2119, F2182, F2213, and F2503 for several different transmission line lengths (5, 7.5, 10, and 15 cm) in addition to finite element analysis of the device's interaction with the RF field that patients will be subjected to for MRI. The testing performed by Exponent, a Good Laboratory Practice-certified outside laboratory, found that our EPR oximetry IRs are MR Conditional. A patient with the device can be safely scanned in an MR system if meeting the following conditions: static magnetic field of 1.5-Tesla or 3-Tesla, maximum spatial field gradient of 4000 G/cm, and maximum MR system reported, whole body averaged SAR of 2.0 W/kg at 1.5 and 3 T. Under these conditions, the IRs are expected to produce a maximum temperature rise of less than 4.0 °C after 15 min of continuous scanning. In non-clinical testing, the image artifact caused by the device was found to extend approximately 0.5 cm from the IR when imaged with a gradient-echo pulse sequence in a 3 T MRI system.

Device geometry and concurrent oxygen measurements

The geometry of the implant has also changed through various iterations, as shown in Fig. 2. When first developed, the geometry of the transmission line was fork-shaped to accommodate being implanted into the small intracranial volume of a mouse brain and to provide simultaneous independent measurements at multiple sites. There were both two and four-sensor models based on this geometry. The transmission line extended down into the tissue-site, directly under the coupling loop.

While this allowed us to acquire multi-site measurements, this form of the device had to be implanted surgically through a large incision and multiple burr holes through the skull. To improve the clinical usefulness and applicability, we converted the geometry into a linear configuration, which allows the device to be placed readily into tissues through a single, small surgical incision. We also minimized the size of the sensor loops to make the device less invasive to minimize perturbing the nearby tissue.

In this linear configuration (see Fig. 2d), the current prototype of the device includes four sensor loops containing oxygen sensitive paramagnetic material along the length of a transmission line. Previous models of the device included one, two and four sensor loops. Because each sensor loop can provide an oxygen measurement at a particular site, we opted to have four loops in the current design. This will provide greater flexibility in the number of measurable locations within the tumor and/or the potential for measuring tissues adjacent to the tumor. In addition to querying multiple locations simultaneously, repeated measurements at each site provides greater knowledge about the heterogeneity and the dynamics of oxygenation of the tissue into which each sensor loop is implanted.

In the current configuration, each sensor loop is separated by 7 mm (measured midpoint to midpoint) to characterize heterogeneity and to facilitate signal detection. We utilize three-dimensional gradient coils in our EPR spectrometer to spectrally and spatially resolve the signals from each of the four sensor loops. However, the analysis of the four EPR signals may be complicated if the phase rotates appreciably along the length. This phenomenon would be apparent if the proximal sensing loop gave an absorption signal while the distal signal had a dispersive shape. This is not something that we have seen in any of our animal studies with the IR. If it became an unforeseen issue in the future, it could be simply addressed by modifying the data fitting routine to assume different phases for the various components along the length of the IR.

Device Encapsulation

The entire four sensor model with a linear configuration and the MP35N transmission line (Fig. 2d) is encapsulated within a medical grade silicone elastomer (A-103 Medical Grade silicone elastomer from Factor II of Lakeside, AZ). The silicone elastomer is a type of polydimethylsiloxane (PDMS), which is oxygen-permeable and is currently used in encapsulating, coating, and in injection molding of various medical implants [18]. PDMS has a dielectric constant of 2.3–2.8 and an electrical conductivity of $4 \times 10^{-23} \Omega\text{m}$. The material does not have an EPR signal, even at x-band frequencies.

The application of this coating has evolved during our studies. Initially, when the IR system was dip-coated in PDMS, we found the probes did not consistently stay embedded in the sensor loops once implanted in animal tissue [17]. However, we have recently found that encapsulating the devices using silicone elastomer to form a cylindrical encasing provides additional mechanical robustness (Fig. 3). In tests performed with chicken muscle tissue, all probes remained embedded in the sensor loops during device implantation and removal. We plan to further monitor the aspect of tissue adhesion disrupting device function in future animal testing.

Since the entire device is encapsulated in PDMS, we needed to ensure radial diffusion of oxygen through the PDMS outweighed the axial diffusion of oxygen along the length of the device between probes (Fig. 4a). The presence of axial diffusion would result in the four probes not providing independent oxygen measurements. To evaluate radial and axial diffusion of oxygen in the IR encapsulated by PDMS, we built a testing chamber shown in Fig. 4b. The two chips on one side of the chamber are in one environment, the “alpha,” while the other two probes are in the “bravo” environment. The two environments are separated by an epoxied wall and the gaseous environments were controllable. The experiment procedure involved measuring the changing linewidth (LW) of the differently coated probes (i.e., glass, PDMS, or no coating) when exposed to different percentages of oxygen (testing metrics shown in Table 1). An incident power of 14 dBm (25 mW) was used throughout the experiment. Session one was designed to evaluate if the chamber had any leaks to the external environment. If the LWs between the two probes in the alpha and bravo environments were not equal when exposed to the same gas, it would indicate that a chamber

Had a leak in it. Session two was designed to evaluate leaks from one chamber into the other chamber. This would be apparent if the two probes exposed to room air demonstrate LWs associated with nitrogen exposure. Session three was a verification of Session two, but it was expected that the LWs from the probes in the alpha chamber would increase when the environmental gas switched from nitrogen to 5% oxygen. Session four was designed to evaluate oxygen diffusion down the length of the IR, as glass is impermeable to oxygen. Therefore, the two chips embedded in glass should not have been influenced by the nitrogen flowing in that chamber and should have demonstrated room air LWs if oxygen was not flowing down the length. Session five was a verification of Session two, but it was expected that the LWs from the probes in the alpha chambers would increase where the oxygen in the environment switches from nitrogen to 5% oxygen. Session six was a repeat of Session one, but involved using a PDMS coated IR. If the chambers worked properly, as determined by the previous sessions, then the LWs associated with all four probes in this session should be similar. The gas was then switched to 5% oxygen in the alpha chamber at the beginning of Session seven. We observed a dynamic response in two of the chips exposed to an increased percent of oxygen and no change in the oxygen response in the environment that remained constant. This proved that there was no detectable axial diffusion between probes, even after a period of 10 min as demonstrated in Fig. 5. Complete data from this experiment are shown in Table 2. A spectral fitting routine was used to calculate the LW errors for each sensor independently. If the errors within a given session are compared, it should be noted that the resulting errors are expected to vary because the signal amplitudes will not always be equivalent across each of the four sensors. Further diversity in the error between sensors arises from four LW measurements being reported from multi-second scans. The error and the LW estimates extracted from each scan is not necessarily a precise representation of the true value, but rather what was measured during that time-period. We felt that reporting on the estimates spanning some longer length of time (13 min) and averaging across each sensor provided the greatest amount of information.

A multi-step fabrication method to achieve consistent device production has also been developed. The dimensions used for our system are based on preserving the device's ability to fit through a biopsy needle or angio-catheter system for future implantation. Based on input from clinical collaborators, we are aiming to keep device dimensions small enough, such that the device fits through an 18 G needle/tubing implantation system. This size needle has been determined based on clinically acceptable sizes for a non-anesthetic procedure. While direct visual placement will be used for implanting the device in the initial proposed site (malignant gliomas) the intent is for the device will to be applicable for other tissues and sites, once it is first determined safe and efficacious in the brain. The device has been designed to fit through a needle by allowing the coupling loop to collapse during implantation; then a small silicone disk can be readily snapped into place over the coupling loop once the device is implanted. The silicone disk would then hold the coil in its geometric circular shape for optimal use in EPR measurements.

Review of evidence to date and overview of next steps

The safety and effectiveness of the IR system has been repeatedly demonstrated in animal studies [14–20]. LiNc–BuO encased by PDMS in a single cylindrical rod implant (the

‘OxyChip’) is currently in an ongoing FDA Phase 1 Trial of Safety and Efficacy. Initial results from the OxyChip are very promising in regard to establishing the safety and efficacy of the encased probes as implants, and the IR’s sensing probes are made from the same material. All the materials used in the IR other than the oxygen-sensing particulates (LiNc–BuO), specifically PDMS, MP35N wire, and the silicone disk (if used in later versions), are already incorporated into clinical care in other FDA approved implanted medical devices.

In all histological analyses, we have seen only a minimal, expected level of chronic inflammation, similar to cellular responses seen when a wire is present in tissue [17, 19, 20]. We have no reason to believe that a further study in human subjects in the context of the IR would produce a different reaction. However, we are pursuing external biocompatibility testing and chemical characterization testing of the IR in accordance with Biological Evaluation of Medical Devices ISO 10993-18 and ISO 10993-12 to further address any safety concerns. The device will be tested for aspects such as cytotoxicity, sensitization, and carcinogenicity in accordance with ISO 10993-5, 10993-10, 10993-11, 10993-3.

In the near future, we will seek an Investigational Device Exemption (IDE) from the FDA to pursue a Phase 1 Trial of Safety and Efficacy for the IR system. The FDA’s guidance on IDE’s states “...early feasibility studies are only appropriate when additional nonclinical testing would not provide the information needed to advance the developmental process [23].” In consonance with this guidance and based on the evidence from past and currently ongoing preclinical studies using the IR system, we believe that an early feasibility study in human subjects is the next appropriate step for evaluating the IR system and to confirm its acceptability for use in human subjects.

Summary

Treatment outcomes for cancer therapy may be improved by tailoring treatment to be delivered at times when oxygen levels are most favorable for treatment-responsiveness, as can be determined by monitoring oxygen levels in the tumors and surrounding tissue. EPR oximetry has been shown to be a method that can be used in clinical practice due to its unique advantages in providing repeatable quantitative measures of tissue oxygen levels. The IR system will extend EPR oximetry measurement’s capability to tumors at any depth.

The current implanted device consists of a transmission line made from MP35N alloy, a biocompatible, FDA-approved wire used in other medical implants. At the end of the device, four paramagnetic sensors are contained within four pre-fabricated sensor loops. From these four sensor loops, four oxygen measurements can be obtained, which allows assessment of heterogeneity of oxygen in the tumor. The geometry of the transmission line is now linear, which will be useful for future implantation into tissue-sites utilizing a biopsy needle. These listed design features have been tested and verified through years of preclinical testing. The device has been further modified to include encapsulation of the silicone elastomer in a cylindrical configuration which will facilitate a smooth entry and removal of the device. This addition has still allowed us to obtain multi-site independent measurements. We have evaluated issues of RF heating by conducting FDA approved Finite Element Method ANSYS HFSS simulations and have also found that our EPR oximetry IRs are MR

Conditional. We have outlined the necessary next steps that we are taking to proceed with an IDE and hope to carry out the first tests of the IR system in humans in early 2017.

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Fig. 1. Wire structure of implantable resonator (IR). Shown in the box are the four sensor loops without the OxyChip material inserted. The sensor loops are 7-mm apart. The circle shown on the left of the device, the coupling loop, is 9 mm in diameter. The total device length, excluding the tail and the coupling loop, is 5 cm (scale shown in cm)

Fig. 2.

Previous iterations of implantable resonator (IR) [10–13]. Panels **a–c** show models with transmission lines made from copper wire. Panels **a** and **b** were used for implantation in the brain, with a non-linear device structure. Panel **c** shows a linear IR made from copper that broke when tested in actively moving tissue in a pig. Panel **d** shows the linear structure of the IR made from MP35N alloy that were dipcoated with a thin layer of PDMS

Fig. 3.
Implantable resonator (IR) encapsulated by a PDMS cylinder. Image obtained with light microscope and is zoomed in to show one sensor loop containing OxyChip material (scale: 1 division = 1 mm)

Fig. 4. Diffusion Schematic and Testing Chamber. Panel **a** is a schematic of radial versus axial oxygen diffusion zoomed-in on one probe on the IR. Panel **b** shows a testing chamber used for the diffusion experiment. Inside the testing chamber is an IR. The testing chamber allowed us to control the inflow and outflow of oxygen gas in the two environments containing two proximal and two distal probes

Fig. 5. Session 7 Diffusion Testing (pO₂ versus time). Panel **a** shows the response for all four probes on one IR. The two chips with the increasing exponential lineshape were in the environment that was switched to a higher percent of oxygen while the two chips with the zero slope pO₂ lineshape remained in a nitrogen environment during the duration of the session. Panels **b–e** show the individual chip responses

Table 1

Diffusion testing setup of IR devices and gases used

Session	Alpha coating	Bravo coating	Alpha gas	Bravo gas
1	None	None	100%N ₂	100%N ₂
2	None	None	100%N ₂	Room air
3	None	None	5%O ₂	Room air
4	PDMS	PDMS/glass capillary	100%N ₂	100%N ₂
5	PDMS	PDMS/glass capillary	5%O ₂	Room air
6	PDMS	PDMS	100%N ₂	100%N ₂
7	PDMS	PDMS	5%O ₂	100%N ₂

Table 2

Diffusion testing results

Session #	Sensor 1 (LW, G)	Sensor 2 (LW, G)	Sensor 3 (LW, G)	Sensor 4 (LW, G)
1	0.3101 ± 0.0047	0.305 ± 0.0092	0.2715 ± 0.0155	0.3474 ± 0.0180
2	n/d	n/d	0.283 ± 0.0022	0.2831 ± 0.0045
3	n/d	n/d	0.6089 ± 0.0137	0.5749 ± 0.0212
4	0.5076 ± 0.0375	0.4035 ± 0.0077	n/d	n/d
5	0.8294 ± 0.037	0.6185 ± 0.0195	n/d	n/d
6	0.3578 ± 0.0065	0.3266 ± 0.0067	0.3494 ± 0.0048	0.3595 ± 0.0243
7	0.6323 ± 0.0071	0.5161 ± 0.0029	0.3096 ± 0.0012	0.2916 ± 0.0067

Note: The linewidths from each probe and each session is shown in the following table after each environment was exposed to the associated session gas for 10 min. If probes demonstrated broad linewidths where the signals superimposed on one another, the linewidths of the individual probes were not determined (n/d)