AWARD NUMBER DAMD17-97-1-7261

TITLE: Non-Invasive Determination of Breast Cancer Oxygen Tension by F-19 NMR and Breast Cancer Physiology in Response to Radiotherapy

PRINCIPAL INVESTIGATOR: Yulin Song

CONTRACTING ORGANIZATION: University of Texas
Southwestern Medical Center
Dallas, Texas 75235-9106

REPORT DATE: August 1999

TYPE OF REPORT: Annual

PREPARED FOR: Commander
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for public release; distribution unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.
Non-Invasive Determination of Breast Cancer Oxygen Tension by F-19 NMR and Breast Cancer Physiology in Response to Radiotherapy

Yulin Song

University of Texas Southwestern Medical Center
Dallas, Texas 75235-9106

The goals of this fellowship are to provide with the solid and extensive training and valuable experience in a modern NMR laboratory for a career as a clinical medical physicist and a breast cancer research scientist to develop and investigate a non-invasive technique of measuring oxygen tension in breast cancer based on 19F MRI of hexafluorobenzene (HFB). In terms of training, I have learned tumor biology, surgical techniques for implanting breast tumors, MRI data acquisitions, digital signal and image processing, and operation of advanced instruments. In terms of research, we found that HFB decays exponentially with a typical biological half-life ranging from 700 to 1200 min. We also found that tumor voxels with high baseline pO2 had different response characteristics from those with initially low pO2 and time constants of well-oxygenated voxels were much shorter than those of hypoxic voxels. A comparison between 19F EPI and NIR showed that the global time constant of pO2 was much longer than that of blood hemoglobin saturation (sO2) and changes in tumor vascular sO2 preceded tumor tissue pO2, particularly for small tumors. Our preliminary results indicated that pO2 and its distribution in tumors changed with tumor growth and there existed heterogeneity in pO2 distribution.

Breast Cancer, Hexafluorobenzene (HFB), Perfluorocarbon (PFC), 19F-NMR, Echo planar imaging (EPI), and NIR spectroscopy
FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

Where copyrighted material is quoted, permission has been obtained to use such material.

Where material from documents designated for limited distribution is quoted, permission has been obtained to use the material.

Citations of commercial organizations and trade names in this report do not constitute an official Department of Army endorsement or approval of the products or services of these organizations.

In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Resources, national Research Council (NIH Publication No. 86-23, Revised 1985).

For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46.

In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

In the conduct of research utilizing recombinant DNA, the investigator(s) adhered to the NIH Guidelines for Research Involving Recombinant DNA Molecules.

In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.

Signature 日期

9-10-99

PI - Date
TABLE OF CONTENTS

Front Cover .................................................................................................................. 1
Report Documentation Page (Standard Form 298) ................................................... 2
Foreword ...................................................................................................................... 3
Table of Contents ....................................................................................................... 4
I. Introduction ............................................................................................................... 5
II. Training Accomplishments .................................................................................... 5
III. Research Accomplishments ................................................................................ 6
IV. Appendices ............................................................................................................ 10
   1) A List of Key Research Accomplishments ....................................................... 10
   2) A List of Reportable Outcomes ....................................................................... 11
   3) Copies of Manuscripts and Abstracts ............................................................... 12
I. INTRODUCTION

The fundamental goals of this predoctoral fellowship are three-fold: 1) to provide me with an opportunity to continue to learn and apply the state of the art NMR technology and radiotherapy techniques to cancer diagnosis and treatment; 2) to provide me with the solid and extensive training, and valuable experience in a modern NMR laboratory for a career as a clinical diagnostics and radiation therapy physicist and a breast cancer research scientist; and 3) to develop and investigate a non-invasive technique of measuring oxygen tension (pO₂) in breast cancers based on ¹⁹F MRI of hexafluorobenzene (HFB), now called the FREDOM (Fluorocarbon Relaxometry using Echo planar imaging for Dynamic Oxygen Mapping). Under the guidance of my mentor, Dr. Ralph P. Mason, I have gone through a rigorous training in tumor biology, surgical techniques for implanting breast tumors, MRI data acquisitions, digital signal and image processing, and using other advanced instruments. In the meantime, I have also done many experiments using both the FREDOM and the near infrared (NIR) spectroscopy techniques to investigate breast tumor oxygenation accompanying respiratory challenges. In addition, I have been developing a new pO₂ mapping software using MS Visual Basic 6.0 and building new MRI/NIR coils. Some of the research results and findings have been presented at various international conferences. In this report, I will briefly describe some of the highlights of my past year's training and research. For further information regarding the research results, please refer to the Appendices.

II. TRAINING ACCOMPLISHMENTS

This project primarily uses an Omega CSI 4.7 T MR system with actively shielded gradients (Acustar™, Bruker Instruments, Inc., Fremont, CA, USA). This system, based on a 40-cm diameter bore horizontal magnet, is located in the Rogers Magnetic Resonance Center and is an NIH Biotechnology Resource Facility. I have immediate access to the system and, on average, used three days per week in the past for either experiments or programming. Now I can operate the system independently for both imaging and spectroscopy. To meet our particular needs, I also modified and wrote some NMR-shell based data acquisition and post-processing programs. At present, I am learning and developing a new program based on our current ¹⁹F PBSR-EPI program. This new program will enable us to acquire pO₂ data based on BOLD (blood oxygen level dependent) effect. The BOLD techniques are completely non-invasive and provide qualitative indications of tumor oxygenation on the basis of magnetic susceptibility gradient changes around vasculature. This will allow us to compare the BOLD results with those obtained using the FREDOM and NIR spectroscopy. This project also involves the investigation of tumor physiology, which requires the use of rat tumors and implanting mammary adenocarcinoma 13762 NF in female Fisher rats is a complicated surgical procedure. Now I have learned the techniques and can do the surgery with confidence. In addition, I have also learned the techniques of blood gas analysis using fiber optic pulse oximeter and how to use automated microelectrode systems. As an important part of my Ph.D. training and research, I have been developing a new pO₂ mapping software using MS Visual Basic 6.0. This is a windows-based software that allows us to perform many special operations on the data.
III. RESEARCH ACCOMPLISHMENTS

It is widely appreciated that tumor oxygenation may significantly influence therapeutic success. In particular, the efficacy of radiotherapy and photodynamic therapy depends on $pO_2$. Separate or combined chemotherapeutic approaches have also been proposed, exploiting selective cytotoxicity of bioreductive drugs towards hypoxic cells, and certain drugs may act preferentially under hypoxic conditions. Many techniques for measuring tissue oxygenation have been developed. However, most of them are either invasive or lack of spatial resolution. NMR techniques have the potential advantages of being non-invasive and repeatable, and having high spatial resolution.

Fluorine NMR can provide a direct measurement of $pO_2$ based on the response of the spin-lattice relaxation rate ($R_1 = 1/T_1$) of perfluorocarbons (PFCs) to $pO_2$ in vivo. We have surveyed the relative sensitivity of several PFCs and now find HFB offers exceptional sensitivity to changes in $pO_2$ with relatively little response to temperature. HFB has a single resonance, providing optimal signal-to-noise ratio (SNR). We have now applied this novel technique to investigate dynamic changes in $pO_2$ in tumors in response to respiratory challenges and the feasibility of mapping the clearance rate of HFB, and compared the results with changes in hemoglobin saturation ($sO_2$) and concentration in tumor vasculature observed using a new NIR system.

Breast 13762 NF or Dunning prostate R3327-AT1 adenocarcinomas were implanted in skin pedicles on the forebacks of adult female Fischer or male Copenhagen rats (∼ 250 g). Once the tumors reached ∼ 1 cm diameter, the rats were anesthetized with 200 µl ketamine hydrochloride, intramuscularly and maintained under general gaseous anesthesia with 33% $O_2$, 66% $N_2O$, and 0.5% methoxyflurane. The tumor blood $sO_2$ was assessed by NIR spectroscopy using a frequency-domain dual wavelength system (758 nm and 782 nm). The tumor blood volume and $sO_2$ were calculated from the light amplitude.

\[
\Delta[Hb]_{total} = -3.63 \log (A_f/A_c)^{758} + 8.68 \log (A_f/A_c)^{782} \\
\Delta[HbO_2] - \Delta[Hb] = -18.49 \log (A_f/A_c)^{758} + 21.20 \log (A_f/A_c)^{782}
\]

where $A_f$ is the initial amplitude (amplitude of baseline), $A_c$ is the current amplitude, and L is the optical pathlength between the source and detector.

Once stable baseline measurements were achieved, the inhaled gas was altered to pure oxygen or carbogen (95% $O_2$ + 5% $CO_2$) and dynamic changes were observed over a period of two hours. Both the magnitude and rate of change of $sO_2$ were examined. Following the NIR experiments, 40 µl HFB were injected directly into both central and peripheral regions of the tumors using a Hamilton syringe with a 32 gauge needle. The animals were placed on its side in a cradle with a thermal blanket to maintain the body temperature at 37°C. A tunable 2 cm $^1H/^{19}F$ single turn solenoid coil was placed around the tumor and MR experiments were performed using the 4.7 T magnet. Tumor dynamic changes in $pO_2$ were monitored using $^{19}F$ PBSR-EPI. Regional tumor $pO_2$ was estimated using the relationship:

\[
pO_2(\text{torr}) = \frac{[R1 - 0.074]}{0.0016},
\]
where $R1$ is the spin lattice relaxation rate of HFB. Twenty-three $pO_2$ maps were produced in 3 hours with respect to respiratory challenges. Clearance ($\tau$) maps were also produced using the EPI images with the longest delays (90 sec).

![Figure 1. $^1H$ and $^{19}F$ coronal images of a breast tumor. FOV = 48 x 48 mm, matrix size = 128 x 64, and slice thickness = 4 mm.](image)

Figure 1 shows conventional spin echo (SE) $^1H$ images (top four) and corresponding $^{19}F$ SE images of a representative breast tumor. $^1H$ images were acquired to show the tumor anatomy and $^{19}F$ images to show the distribution of HFB within the tumor. Comparison of the $^1H$ and $^{19}F$ images reveals that the tumor was centrally labeled in this case. For many regions, the HFB signal intensity was found to decay exponentially with a typical biological half-life ranging from $T_{1/2} = 700$ to 1200 min. Since HFB is a non-ionic freely diffusible tracer, we believed that clearance rate would provide an indication of relative tumor blood flow (TBF).

![Figure 2. T1 relaxation curves of a well-oxygenated voxel (a) and a hypoxic voxel (b).](image)

Good T1 relaxation curve fit is crucial in $^{19}F$ PBSR-EPI oximetry. To assess the goodness of curve fit, I wrote a Unix based NMR-shell script program to display the T1 relaxation curves of individual voxels of the PBSR-EPI images. The graphical representation of the data,
relaxation model, and goodness of fit provides a quick and convenient way of assessing the
goodness of fit provides a quick and convenient way of assessing the
quality of the data. Figure 2 shows T1 relaxation curves of a well-oxygenated voxel (a) and a
hypoxic voxel (b).

![Dynamic Changes in pO2 of 44 Specific Voxels](image)

**Figure 3.** Dynamic changes in pO2 of 44 specific voxels of a breast tumor.

The $^{19}$F MR-EPI oximetry of tumor has the distinct advantage over other techniques that
subsequent measurements are completely non-invasive. The greatest strength of this method is
the ability to trace the fate of individual voxels (regions) with respect to therapeutic
interventions. Figure 3 shows dynamic changes in $pO_2$ of 44 specific voxels of a breast tumor
with respect to different inhaled gases. Error bars have been omitted for the sake of clarity. It is
noteworthy that voxels with high baseline $pO_2$ had significantly different response characteristics
from those with initially low $pO_2$, which showed small changes. To further investigate the
temporal response of individual voxels, we modeled the temporal response in $pO_2$ using
exponential equations:

1) $y = a + b \cdot (1 - e^{-t/\tau})$, for increasing trend
2) $y = a + b \cdot e^{-t/\tau}$, for decreasing trend

where $y$ is $pO_2$, $a$ and $b$ are two constants, $t$ is time, and $\tau$ is the time constant.

We found that the time constants of well-oxygenated voxels (10 ~ 20 min) were much
shorter than those of hypoxic voxels (> 50 min) and the global $pO_2$ time constant (60 ~ 80 min)
was much longer than the blood hemoglobin saturation ($sO_2$) time constant (10 ~ 20 min) (for
detailed information regarding the NIR results, please refer to Appendices). NIR showed
significant changes in tumor vascular oxygenation accompanying respiratory interventions. $^{19}$F
MR-EPI showed significant changes in tumor tissue $pO_2$, with considerable regional
heterogeneity in both absolute values and rate of change accompanying interventions. In general,
changes in tumor vascular $sO_2$ preceded tumor tissue $pO_2$, particularly for smaller tumors. Figure 4 shows the exponential response model of a representative voxel when the inhaled gas was switched from 33% $O_2$ to carbogen.

![Figure 4. Dynamic response of $pO_2$: the time constant $\tau$ of a representative voxel.](image)

We also found that strong correlation existed between the maximum $pO_2$ value (breathing carbogen) attained during the course of an experiment and mean baseline $pO_2$ ($r^2 = 0.7856$) (Figure 5a) and between mean $pO_2$ (breathing carbogen) and mean baseline $pO_2$ ($r^2 = 0.9065$) (Figure 5b).

![Figure 5. Correlation between maximum $pO_2$, mean $pO_2$ (breathing carbogen) and mean baseline $pO_2$.](image)

Currently, I am still in a process of analyzing the data. However, preliminary results indicate that this method offers several distinct advantages: 1) requisite precision of $pO_2$ determinations; 2) ability to interrogate selected tumor regions; 3) high temporal resolution; 4) low sensitivity to temperature; and 5) small volumes of reporter molecule. Now, I am building new RF coils that allow us to measure tumor tissue $pO_2$ using $^{19}$F MR-EPI and vascular $sO_2$ using NIR spectroscopy simultaneously. This technique will further enhance our understanding of tumor physiology and response to therapy.
IV. APPENDICES

1. A List of Key Research Accomplishments

- The HFB signal intensity was found to decay exponentially with a typical biological half-life ranging from $T_{1/2} = 700$ to 1200 min, which, we believe, would provide an indication of relative tumor blood flow (TBF).

- Tumor voxels with high baseline $pO_2$ had significantly different response characteristics from those with initially low $pO_2$, with voxels of high baseline $pO_2$ showing significant changes in $pO_2$ while voxels of low baseline $pO_2$ showing small changes.

- Time constants ($\tau$) of well-oxygenated voxels (10 ~ 20 min) were much shorter than those of hypoxic voxels (> 50 min). The global $pO_2$ time constant (60 ~ 80 min) was much longer than the blood hemoglobin saturation ($sO_2$) time constant (10 ~ 20 min).

- NIR spectroscopy showed significant changes in tumor vascular oxygenation ($sO_2$) accompanying respiratory interventions. $^{19}$F MR-EPI showed significant changes in tumor tissue $pO_2$, with considerable regional heterogeneity in both absolute values and rate of change accompanying interventions. Changes in tumor vascular $sO_2$ preceded tumor tissue $pO_2$, particularly for smaller tumors.

- Strong correlation existed between the maximum $pO_2$ value attained during the course of an experiment and mean baseline $pO_2$ ($r^2 = 0.7856$) and between mean $pO_2$ and mean baseline $pO_2$ ($r^2 = 0.9065$).

- $^{19}$F EPI oximetry of HFB was proven to be a useful technique for measuring tumor oxygenation.

- $pO_2$ and the distribution of $pO_2$ in breast 13762 NF and Dunning prostate R3327-AT1 adenocarcinomas changed with tumor growth and there existed heterogeneity in $pO_2$ distribution.

- Tumor oxygenation could be manipulated by inhaling different gases.
2. **A List of Reportable Outcomes**

Manuscripts:

1) "Tumor Oximetry: A Comparison between Near-infrared Frequency-Domain Spectroscopy of Hemoglobin Saturation and $^{19}$F MRI of Hexafluorobenzene"
Katherine L. Worden, **Yulin Song**, Xin Jiang, Anca Constantinescu, Ralph P. Mason, and Hanli Liu
(Presented at the International Symposium on Biomedical Optics, sponsored by the Society of Photo-Optical Instrumentation Engineers (SPIE), 1998, in press).

2) "Tumor Oximetry: Comparison of $^{19}$F MR EPI and Electrodes"
Ralph P. Mason, Sandeep Hunjan, Anca Constantinescu, **Yulin Song**, Dawen Zhao, Eric W. Hahn, Peter P. Antich, and Peter Peschke
(Presented at the 27th Annual Meeting of International Society on Oxygen Transport to Tissue (ISOTT), 1999, in press).

Abstracts:

1) "Regional Tumor Oxygen Tension and Blood Flow: Correlation Studies Using 19F PBSR-EPI of Hexafluorobenzene"
**Yulin Song**, Ralph P. Mason, Sandeep Hunjan, Anca Constantinescu, Eric Hahn, and Peter Antich

2) "Tumor Oxygen Dynamics: Comparison between $^{19}$F MR-EPI of Hexafluorobenzene and Frequency Domain NIR Spectroscopy"
**Yulin Song**, Kate L. Worden, Xin Jiang, Dawen Zhao, Anca Constantinescu, Hanli Liu, and Ralph P. Mason
(Presented at the 27th Annual Meeting of International Society on Oxygen Transport to Tissue (ISOTT), 1999).

3) "Tumor Oxygenation and Measurement of Regional Dynamic Changes"
Ralph P. Mason, Sandeep Hunjan, Anca Constantinescu, **Yulin Song**, Eric W. Hahn, Peter P. Antich, Christian Blum, and Peter Peschke
(Presented at International Conference on Molecular Determinants of Sensitivity to Antitumor Agents, sponsored by American Association for Cancer Research (AACR), 1999).
3) Copies of Manuscripts and Abstracts
Tumor Oximetry: A comparison between near-infrared frequency-domain spectroscopy of hemoglobin saturation and $^{19}$F MRI of hexafluorobenzene

Katherine L. Worden*, Yulin Song†, Xin Jiang*, Anca Constantinescu†, Ralph P. Mason†, Hanli Liu*

*Joint Biomedical Engineering Program
University of Texas at Arlington/University of Texas Southwestern Medical Center at Dallas
Dallas, TX 75235

†Department of Radiology
University of Texas Southwestern Medical Center at Dallas
Dallas, TX 75235

ABSTRACT

Studies have shown that hypoxic tumor cells are relatively more resistant to radiotherapy, chemotherapy, and photodynamic therapy. Tumor oximetry, e.g., measurement of oxygen tension (pO$_2$) of tissue and/or blood oxygenation (SO$_2$) of the vascular bed, could be valuable for optimizing treatment plans.

In this study, we employed a recently developed homodyne system to measure changes in hemoglobin saturation (SO$_2$) and concentration in the vascular bed of rat prostate and breast tumors. For comparison, tissue pO$_2$ values were measured using $^{19}$F MR EPI of hexafluorobenzene, providing a map of regional tumor oxygenation tension. Both SO$_2$ and pO$_2$ measurements were taken while the inhaled gas was alternated between 33% oxygen, 100% oxygen and carbogen (95% oxygen, 5% CO$_2$).

The results obtained for both techniques showed significant changes in tumor oxygenation accompanying respiratory challenge, with changes in vascular SO$_2$ preceding tissue pO$_2$ change. The combined use of these two techniques provides new insight into the dynamics of tumor oxygenation by making available a method of obtaining regional information of the state of the tissue, as well as a non-invasive, real-time method for determining changes in the vascular bed.

Keywords: Frequency-Domain Spectroscopy, NIR spectroscopy, $^{19}$F MRI, Hexafluorobenzene, Oximetry

1. INTRODUCTION

Frequently, blood vessel formation is unable to keep up with the rapid growth of a tumor. If this occurs, the cells in the tumor furthest from a fresh blood supply will suffer a lack of oxygen and hypoxic areas will form (chronic hypoxia). These regions can be as much as 3 times more resistant to radiotherapy. In addition to studies in vitro and in animal tumors, there is increasing evidence from clinical trials that poorly oxygenated tumors indicate poor prognosis for patients. Methods of determining the oxygen content of a tumor could, therefore, be helpful in the development of an optimal treatment plan. This paper will present the experimental results of two such methods: NIR spectroscopy to determine blood oxygenation (SO$_2$) of the tumor’s vascular bed and $^{19}$F MRI of hexafluorobenzene (HFB) to determine tissue pO$_2$.

NIR spectroscopy, through use of a recently developed frequency-domain system, based on an in-phase and quadrature (IQ) demodulator chip, is attractive as a non-invasive, inexpensive, portable, real-time system that can provide global SO$_2$ values. We show that this IQ system can be used to determine the SO$_2$ in a tumor’s vascular bed and measure the
response of blood volume and oxygen saturation to inhaled gas. The technique of using \( ^{19}F \) MRI relaxometry to map tissue \( pO_2 \) is also relatively new.\(^5\) The spin-lattice relaxation rate of hexafluorobenzene is particularly sensitive to oxygen while being insensitive to temperature.\(^6\) Following direct injection of HFB into a tumor, \( ^{19}F \) MRI maps tissue \( pO_2 \) at millimeter resolution. This method facilitates measurements of dynamic changes in \( pO_2 \) accompanying therapeutic interventions and allows the fate of individual voxels to be traced.

Through comparison of these two techniques, it is possible to examine the relationship between \( SO_2 \) of the vascular bed and \( pO_2 \) of the tissue. Blood oxygenation, blood volume, arterial \( SO_2 \) and temperature may also be compared.

2. METHODS AND INSTRUMENTATION

2.1 Tumor Model

Dunning prostate adenocarcinoma R3327-AT1 was implanted in adult male Copenhagen rats and NF13762 breast tumor in female Fisher rats. The tumors were grown in pedicles\(^7\) on the forebacks of the rats until they were approximately 2 cm in diameter. Rats were anesthetized with 200 μl ketamine hydrochloride (100 mg/ml) and maintained under general gaseous anesthesia with 33 % inhaled \( O_2 \) [0.3 dm\(^3\)/min \( O_2 \), 0.6 dm\(^3\)/min \( N_2 \), and 0.5% methoxyflurane] through a mask placed over the mouth and nose. Body temperature was maintained with a warm water blanket. A fiber optic pulse oximeter was placed on the hind foot to monitor arterial oxygenation (\( A_{SO_2} \)) and a fiber optic probe was inserted rectally to measure temperature. Inhaled gas was alternated between 33% oxygen, 100% oxygen and carbogen (95% oxygen, 5% carbon dioxide). NIR and EPI measurements were performed sequentially for comparison.

2.2 NIR Spectroscopy

As shown in figure 1, we used a new homodyne system able to determine amplitude and phase changes of light.\(^4\) In this setup, an RF source modulates the light from two laser diodes (wavelengths 758 nm and 782 nm) at 140 MHz. The light passes through fiber optic cables, is transmitted through the tumor tissue, and is collected by a second fiber bundle. The light is then detected by a PMT and is demodulated with a commercially available in-phase and quadrature (IQ) demodulator chip into I and Q components. Once these components are put through a low pass filter, they can be used to calculate amplitude and phase changes caused by the tumor. These steps can be seen mathematically in equations 1-4.

\[
\begin{align*}
(1) & \quad I(t) = 2A \sin(\omega t + \theta) \sin(\omega t) = A \cos(\theta) - A \cos(\omega t + \theta) \quad \text{--- low pass filter ---} I_{dc} = A \cos(\theta) \\
(2) & \quad Q(t) = 2A \sin(\omega t + \theta) \cos(\omega t) = A \sin(\theta) + A \sin(\omega t + \theta) \quad \text{--- low pass filter ---} Q_{dc} = A \sin(\theta) \\
(3) & \quad \theta = \tan^{-1}(Q_{dc}/I_{dc}) \\
(4) & \quad A = (I_{dc}^2 + Q_{dc}^2)^{1/2}
\end{align*}
\]

\( A = \) amplitude of detected light; \( \theta = \) phase of detected light; \( \omega = \) modulation frequency (140 MHz)

![Figure 1: Setup for NIR experiment.](image-url)
NIR spectroscopy can be used to determine hemoglobin saturation because the extinction coefficient values of deoxygenated hemoglobin differ from those of oxygenated hemoglobin at the wavelengths selected (758 nm and 782 nm). At this point in our algorithm calculations, we have assumed background absorbance to be negligible and estimated that the absorption coefficients were composed of the extinction coefficients for deoxy-hemoglobin and oxy-hemoglobin multiplied by their respective concentrations (equations 5&6).

\[
\begin{align*}
\mu_{a_{758}} &= \varepsilon_{\text{Hb}_{758}}[\text{Hb}] + \varepsilon_{\text{HbO}_2_{758}}[\text{HbO}_2] \\
\mu_{a_{782}} &= \varepsilon_{\text{Hb}_{782}}[\text{Hb}] + \varepsilon_{\text{HbO}_2_{782}}[\text{HbO}_2]
\end{align*}
\]

The IQ system does give both phase and amplitude values, but given the tumor’s small size and our fiber configuration, we haven’t yet derived a suitable algorithm to compute \( \mu_a \) and \( \mu_t \). The data presented in this paper were analyzed using Beer-Lambert’s law and the amplitude values to find trends in the changing absorption coefficients (equation 7). By manipulating equations 5-7, we can calculate changes in blood volume and saturation from the transmitted amplitude of the light through the tumor (equations 8&9).

\[
\begin{align*}
\mu_{ac} - \mu_{at} &= 1/L \cdot \log (A_t/A_c) \\
\Delta[Hb]_{\text{initial}} &= -3.63 \cdot \log (A_t/A_c)_{758} + 8.68 \cdot \log (A_t/A_c)_{782} \\
\Delta[HbO_2] - \Delta[Hb] &= -18.49 \cdot \log (A_t/A_c)_{758} + 21.20 \cdot \log (A_t/A_c)_{782}
\end{align*}
\]

\( A_t \) = initial amplitude (amplitude of baseline); \( A_c \) = current amplitude; \( L \) = optical pathlength between source/detector; The constants were computed with extinction coefficients for oxy- and deoxy- hemoglobin at the two wavelengths used.

### 2.3 MRI Instrumentation and Procedure

MRI experiments were performed on an Omega CSI 4.7 T 40 cm system with actively shielded gradients. A homebuilt tunable \(^1\text{H}/^{19}\text{F}\) single turn solenoid coil was placed around the tumor. HFB (40 µl) was administered directly into the tumor using a fine sharp (32 G) needle with deliberate dispersion along several tracks to interrogate both central and peripheral tumor regions. HFB is ideal for the imaging of pO\(_2\) because it has a single resonance and its relaxation rate varies linearly with oxygen concentration. \(^1\text{H}\) images were acquired for anatomical reference using a traditional 3D spin-echo pulse sequence as seen in figure 2a. Conventional \(^{19}\text{F}\) MR images (figure 2b) were then taken to show the 3D spin-echo pulse sequence of the HFB in the tumor. Figure 2b may be directly overlaid over figure 2a to show the position of the HFB in that slice.

**Figure 2a:** Proton \(^1\text{H}\) coronal image of a representative slice through a breast tumor (NF13762). TR = 250 ms, TE = 8 ms, NA = 2, FOV = 48 x 48 mm, slice thickness = 4 mm, and matrix size = 128 x 64 x 8.

**Figure 2b:** Corresponding \(^{19}\text{F}\) MR Image showing distribution of HFB within the tumor. TR = 150 ms, TE = 8 ms, FOV = 48 x 48 mm, matrix size = 128 x 64x8.
Tumor oxygenation was assessed using \( ^{19}\text{F} \) PBSR-EPI of HFB. The PBSR preparation pulse sequence consists of a series of 20 non-spatially selective saturating 90° pulses with 20 ms spacing to saturate the \( ^{19}\text{F} \) nuclei. Following a variable delay time \( \tau \), a single spin echo EPI sequence with “blipped” phase encoding was applied. A PBSR-EPI image corresponding to the images shown in figures 2a and 2b is shown in figure 2c. Fourteen 32x32 PBSR-EPI images, with \( \tau \) ranging from 200 ms to 90 sec and an FOV of 40x40 mm, were acquired in eight minutes. An R1 map was obtained by fitting signal intensity of each voxel of the fourteen images to a three parameter relaxation model by Levenberg-Marquardt least squares algorithm (equation 10):

\[
\gamma_n(i,j) = A(i,j) \cdot [1 - (1 + W) \cdot \exp(-R1(i,j) \cdot \tau_n)] \\
(n = 1, 2, \ldots, 14) \\
(i, j = 1, 2, \ldots, 32)
\]

where \( \gamma_n(i,j) \) is the measured signal intensity corresponding to delay time \( \tau_n \) (the \( n \)th images) for voxel \( (i,j) \), \( A(i,j) \) is the fully relaxed signal intensity amplitude of voxel \( (i,j) \), \( W \) is a dimensionless scaling factor allowing for imperfect signal conversion, and \( R1(i,j) \) is the relaxation rate of voxel \( (i,j) \) in unit of sec\(^{-1} \). A, W and R1 are the three fit parameters.

Figure 2c: \( ^{19}\text{F} \) PBSR-EPI projection image obtained from the tumor in figure 1 in a single acquisition (\( \tau = 90 \) s). Fourteen images were acquired with variable relaxation delays (\( \tau \)) ranging from 200 ms to 90 sec. Using a 32 x 32 matrix, FOV of 40 x 40 mm, pO\(_2\) maps were generated with 1.25 x 1.25 mm resolution.

\( pO_2 \) maps were then generated by applying the calibration curve: \( pO_2 \) (torr) = \([R1(s^{-1}) - 0.074]/0.0016\) to the R1 maps. The map shown in figure 2d focuses on a region of the same slice that was presented in figures 2a-2c.

Figure 2d: Typical pO\(_2\) map, composed of fourteen PBSR images from the tumor presented in figure 2a-c. Using a 32 x 32 matrix, FOV of 40 x 40 mm, pO\(_2\) maps were generated with 1.25 x 1.25 mm resolution.
3. RESULTS

3.1 NIR Results

The effects of the inhaled gas on hemoglobin saturation and concentration, as recorded by the IQ system, are shown below in figures 3a & 3b. The X-axis shows time in minutes from the start of the experiment and the dotted vertical lines mark the point when the gas was changed. Hemoglobin saturation and concentration are presented as unit-less, relative trends. It can easily be seen that hemoglobin saturation begins to increase almost immediately after a gas switch from baseline (33% oxygen) to either carbogen or 100% oxygen and increases steadily for several minutes. Total hemoglobin change is quite small in comparison, indicating relatively constant blood volume in the tumor. These trends seem fairly consistent for both breast and prostate tumors. Typical responses of a breast tumor and prostate tumor are presented in figures 3a & 3b, respectively.

![Figure 3a](image)

**Figure 3a:** Hemoglobin saturation and concentration change in a breast tumor.

![Figure 3b](image)

**Figure 3b:** Hemoglobin saturation and concentration change in a prostate tumor.
It is also worthwhile to compare the saturation changes measured in the tumors vascular bed by the IQ system with the arterial saturation changes measured by a pulse oximeter from the rat's hind foot. One such comparison taken from a prostate tumor is presented below as figure 4. Hemoglobin saturation in the vascular bed is again represented as a unit-less trend and arterial saturation values are presented to the right. Again, the X-axis gives the time from the beginning of the experiment in minutes and the dotted lines mark the time of gas change. In this case, the arterial saturation follows the same trend as the tumor vascular bed's hemoglobin saturation, but shows a faster change. Such close similarity wasn't always observed.

**Figure 4:** In the prostate tumor presented in Figure 4, both arterial SO2 and the SO2 in the tumor increased for inhaled carbogen and 100% O2 and decreased for 33% O2. Arterial SO2 in hind foot measured by a commercial pulse oximeter and SO2 in tumor using IQ system.

### 3.2 MRI Results

MRI provides the advantage of being able to look at regional changes in pO2 values. Histograms, such as those presented in figure 5, are able to show the heterogeneity of pO2 values within the tumor as well as the average pO2 values. The data presented here were taken from a breast tumor and show the average values from the data of several pO2 maps that were taken throughout the administration of each gas. In figure 5a, we see that when the rat was breathing 33% oxygen, the average pO2 value was about 40 torr. When the rat was breathing carbogen (figure 5b), there was a large shift towards higher pO2 values leading to a mean value of about 99 torr. These values increased further while the rat was breathing 100% oxygen (figure 5c) such that the average voxel now had a pO2 value of about 145 torr. The time course of these changes is presented later in the paper in figure 7b.
Figure 5a:
pO$_2$ range while the rat was breathing 33% oxygen.  
Average value = 40 ± 3 torr

Figure 5b:
pO$_2$ range while the rat was breathing carbogen.  
Average value = 99 ± 4 torr

Figure 5c:
pO$_2$ range while the rat was breathing 100% oxygen.  
Average value = 145 ± 4 torr
3.3 Comparison

While absolute pO$_2$ values are important for investigating hypoxia, dynamic changes may be more interesting for investigation of response to intervention. In figures 6 & 7, the dynamic changes in pO$_2$ and SO$_2$ are compared. These plots show that pO$_2$ reacts in a very similar fashion to blood saturation, but that the effect is slower. Since the IQ system provides a global measure across the whole tumor, pO$_2$ measurements are presented here as mean values attained from the pixels of each pO$_2$ map. The NIR and MRI data shown are for the same rat undergoing the same procedure, but on consecutive days. Data from two breast tumors of various size are presented. The larger tumor was about 2.1 x 2.4 x 2.2 cm whereas the smaller tumor was 1.9 x 2.2 x 2 cm. Inhaling carbogen and 100% oxygen each consistently resulted in increased SO$_2$ and pO$_2$ over 33% oxygen. The pO$_2$ and SO$_2$ measurements were plotted against the same X-axis to allow rate of change to be compared.

Figure 6a: Saturation from IQ

Figure 6b: pO$_2$ from MRI

Figure 6: These two sets of data were taken from the small rat breast tumor on consecutive days. Both the pO$_2$ and blood saturation increased with a transition from 33% to carbogen, and decreased when switched back to 33%.
Figure 7: These two sets of data were taken from the large rat breast tumor on consecutive days. Both the \( pO_2 \) and blood saturation increased with a transition from 33% to carbogen, and then further increased slightly when switched from carbogen to 100% oxygen. Values began to decrease when switched back to 33%.

To further study the temporal response, the changes in \( pO_2 \) and \( SO_2 \) were modeled using the exponential equations 11 and 12. A non-linear curve fitting method was used to obtain \( \tau \).

\[
\begin{align*}
(11) & \quad \text{For increasing values: } \text{Saturation} = a + b(1-e^{-t/\tau}) \\
(12) & \quad \text{For decreasing values: } \text{Saturation} = a + b(e^{-t/\tau})
\end{align*}
\]

Generally, blood saturation effects had a much shorter time constant than oxygen tension in the tissue (Figure 8). For the larger tumor, the rate of increase and decrease were much faster for \( SO_2 \) than \( pO_2 \). Less difference was seen in the smaller tumor. Further study for confirmation is underway.
Figure 8: Time constants for the data presented in Figures 6&7.

4. CONCLUSION

The data suggest interesting correlations between several physiological parameters. Both tumor vascular Hb saturation and mean pO₂ increased in response to inhaling an elevated percent O₂, either through carbogen or 100% O₂. Arterial SO₂ and tumor SO₂ respond similarly to changes in inhaled gas, with arterial changes preceding changes in the tumor vascular bed. Changes in SO₂ are generally faster than pO₂, though absolute values are highly variable and suggest heterogeneity amongst the tumor population. Given the distinct heterogeneity among tumors even of a given type and size, further investigations are required to form a sound picture of the interplay of multiple physiological parameters.

ACKNOWLEDGEMENTS

This work was supported in part by The Whitaker Foundation (HL, RPM), The American Cancer Society RPG-97-116-010CCE (RPM), and the Department of Defense Breast Cancer Initiative BC962357 (YS). MRI experiments were performed at the Mary Neil & Ralph B. Rogers MR Center, an NIH BRTP Facility no. 5-P41-RR02584. We are very grateful to Sandeep Hunjan for assistance with data analysis.

REFERENCES

TUMOR OXIMETRY: COMPARISON OF $^{19}$F MR EPI AND ELECTRODES

Ralph P. Mason, Sandeep Hunjan, Anca Constantinescu, Yulin Song, Dawen Zhao,
Eric W. Hahn, Peter P. Antich, and Peter Peschke$^+$. 

U.T. Southwestern Medical Center, Dallas, TX and $^+$DKFZ, Heidelberg, Germany

*address correspondence to:

Ralph P. Mason, Ph.D., C. Chem.,
Department of Radiology,
U.T. Southwestern Medical Center,
5323 Harry Hines Blvd.,
Dallas, TX 75235-9058
Tel: (214) 648-8926
Fax (214) 648-2991
E. mail: Ralph.Mason@email.swmed.edu
ABSTRACT

We recently described a novel approach to measuring regional tumor oxygen tension using $^{19}$F pulse burst saturation recovery NMR echo planar imaging relaxometry of hexafluorobenzene. We have now compared oxygen tension measurements in a group of size matched Dunning prostate rat tumors R3327-AT1 made using this FREDOM (Fluorocarbon Relaxometry using Echo planar imaging for Dynamic Oxygen Mapping) approach with a traditional polarographic method: the Eppendorf Histograph. We also compare MR and electrode approaches to monitoring dynamic changes with respect to interventions and demonstrate extension of the MR technique to rat breast tumors.

Key words: echo planar imaging, electrode, MRI, oxygen, prostate, tumor

Abbreviations ARDVARC (Alternated Relaxation Delays with Variable Acquisitions to Reduce Clearance effects); EPI (echo planar imaging); FREDOM (Fluorocarbon Relaxometry using Echo planar imaging for Dynamic Oxygen Mapping); HFB (hexafluorobenzene); i.t (intra tumoral)

Acknowledgments

This work was supported in part by The American Cancer Society (RPG-97-116-010CCE; RPM), DOD Breast Cancer initiative (YS), Verein zur Forderung der Krebserskennung and Krebshandlung e.V. Heidelberg (PP) and the NIH BRTP Facility #5-P41-RR02584.
Introduction

It is widely appreciated that tumor oxygenation may significantly influence therapeutic success. In particular, the efficacy of radiotherapy [1], photodynamic therapy [2] and hypoxia selective chemotherapeutic agents [3] depend on pO2. It had been suggested that the ability to measure tumor oxygenation in patients could allow therapy to be individualized and optimized [4], and indeed, several recent studies have found significant prognostic value based on the Eppendorf Histograph in assessing clinical tumors [5-7]. While electrodes may be considered a "gold standard", they have certain shortcomings and there is clearly a need for alternative methods [8]. We have been developing a new approach based on 19F NMR of perfluorocarbons [9-12] and believe the method can now provide useful measurements of tumor oxygen dynamics in vivo.

The FEDOM approach exploits the exceptional response of the 19F NMR spin lattice relaxation rate, R1, of fluorocarbons to changes in oxygen tension. Fluorocarbons act as ideal liquids and solvation of gases is directly proportional to the partial pressure of the gas (Henry's law). Since oxygen (O2) is paramagnetic it induces relaxation in solution directly proportional to the concentration of oxygen, and hence, pO2 [13]. The highly hydrophobic nature of fluorocarbons ensures both a high solubility of gases, providing molecular amplification, and minimal solvation of other materials (e.g., metal ions) minimizing interference from other environmental factors. We, and others, have explored the use of numerous PFC reporter molecules and various routes of administration [14]. We believe that direct intra tumoral (i.t.) injection of HFB provides an optimal approach to tumor oximetry, and should provide measurements comparable to those obtained using electrodes. In addition, this minimally invasive approach facilitates mapping of dynamic changes in pO2 with respect to interventions.

Methods

Dunning prostate adenocarcinomas (R3327-AT1) were implanted in male Copenhagen rats (~250 g), as described in detail previously [15]. Tumors were divided
into two groups and allowed to grow to about \(~2\) cm\(^3\) or \(>3.5\) cm\(^3\) volume. For MR investigations rats were placed under general gaseous anesthesia with 33% inhaled O\(_2\) (0.3 dm\(^3/min\) O\(_2\), 0.6 dm\(^3/min\) N\(_2\)O, and 0.5% methoxyflurane. Hexafluorobenzene (25 - 40 \(\mu\)l) was injected directly into the tumors in both central and peripheral regions using a Hamilton syringe with a custom made fine sharp needle (32 G). A fiber optic probe was placed rectally to monitor core temperature. NMR experiments were performed using an Omega CSI 4.7 Tesla horizontal bore magnet system with actively shielded gradients with a tunable \(^1\)H/\(^19\)F single turn size-matched solenoid coil placed around the tumor. Following traditional imaging to establish the distribution of HFB, tumor oxygenation was estimated on the basis of \(^19\)F PBSR EPI relaxometry of the HFB [10] with a typical 1.25 mm in plane resolution. For initial work three consecutive R1 measurements were made over a period of 1 hour to investigate reproducibility, and stability of the system when the rats breathed 33% O\(_2\) (baseline). Since R1 is a linear function of pO\(_2\) at constant temperature, pO\(_2\) was estimated on a voxel by voxel basis using the relationship pO\(_2\) (torr) = \((R1 - 0.074)/0.0016\) [10]. The inhaled gas was then altered to 100% oxygen, and relaxation measurements (three) were immediately repeated over a period of 1 hour. Finally, the gas was switched back to the baseline state and three further pO\(_2\) determinations were immediately performed over 1 hour. Our initial studies required 20 mins to produce a pO\(_2\) map, but more recent introduction of the ARDVARC acquisition protocol [12] provides enhanced maps in 8 mins. Breast 13762 NF adenocarcinomas were examined similarly.

Histography was applied to groups of size matched tumors, which did not receive HFB. Halothane was used in place of methoxyflurane. Using the Eppendorf Histograph 100 to 200 individual pO\(_2\) determinations were made in each tumor, as recommended by the manufacturer. For dynamic measurements a Diamond General micro-electrode (700 \(\mu\)m) was inserted to a specific location. Baseline pO\(_2\) was measured and the inhaled gas altered to 100% O\(_2\) or carbogen (95%O\(_2\)/5%CO\(_2\)) for 30 mins. At this stage pO\(_2\) was
again measured. Following a series of measurements with different gases at one location, the needle was moved and the gases cycled again.

Statistical significance of changes in oxygenation was assessed using analysis of variance (ANOVA) on the basis of Fisher PLSD. Experiments were approved by the Institutional Animal Care and Advisory Committee conducted in accordance with National Laws.

Results

Both FREDOM and electrode methods indicated similar oxygen tension distributions for the AT1 tumors (Fig. 1). Moreover, both techniques showed that tumors with volume > 3.5 cm\(^3\) were significantly (p < 0.0001) less well oxygenated than smaller tumors (volume < 2 cm\(^3\)). For the large tumors FREDOM indicated median pO\(_2\) = 2 torr and fraction < 10 torr (HF\(_{10}\)) = 82 %, while the Eppendorf electrode indicated median pO\(_2\) = 3 torr and HF\(_{10}\) = 84%. For the small tumors the match was less good with median = 15 v 8 torr and HF\(_{10}\) = 44 versus 66% for FREDOM and electrode, respectively. Examination of the MR images showed that for 1 small tumor most of the HFB resided very close to the tumor edge and may have biased the apparent pO\(_2\). Indeed, if this tumor was excluded there was no significant difference between the respective pO\(_2\) distributions.

Using the FREDOM approach we also examined response to respiratory challenge. Increasing the concentration of inspired oxygen from 33% to 100% O\(_2\) produced a significant increase (p < 0.0001) in tumor oxygenation for the group of small tumors. In contrast no change was observed in the mean pO\(_2\) for the group of large tumors. A strength of the FREDOM approach is the ability to follow individual tumor regions, with respect to intervention, in this case respiratory challenge. Six representative regions were selected from a single tumor (Fig. 2a). Three regions, which were initially well oxygenated (pO\(_2\) > 10 torr) showed rapid and significant increases within 8 minutes of switching from 33% O\(_2\) to 100% O\(_2\). Changes in relatively poorly oxygenated regions
were much slower, although 2 of 3 regions did show a significant change in pO2 after 24 mins.

Electrode investigation of dynamic changes in pO2 also showed 3 of six regions with significant changes in switching from 33% O2 to 100% O2, but only 1 region was also significantly different with carbogen (Fig. 2b).

In a representative large breast tumor (~4 cm³) we found significant changes in pO2 (p < 0.0001) with respect to respiratory challenge with baseline mean pO2 = 40±3 rising to mean pO2 = 99±4, when rat inhaled carbogen and mean pO2 = 145 ±4 for oxygen inhalation.

Discussion

These results demonstrate the similarly of measurements obtained using traditional electrodes or the new FREDOM approach to tumor oximetry. In each case there was a significant difference in pO2 observed in small versus large AT1 tumors. For larger tumors the hypoxic fraction, mean and median were very similar, together with the range of typical pO2 values. In smaller tumors MR suggested a larger range with a number of measurements in excess of 100 torr. This may have arisen from measurements close to the tumor periphery, which are less common using electrodes.

A significant strength of the FREDOM approach is the ability to monitor dynamic changes in regional pO2 in response to acute interventions. Others have used the Eppendorf system to examine acute changes [16], but this required reintroduction of the needle electrode and generation of new tracks. Not only was this invasive, but it also led to sampling of parallel tissue regions rather than the fate of specific regions. Given the extensive heterogeneity encountered in tumors and steep local gradients in pO2 we believe it will be valuable to follow individual tumor regions. Historically, regional response to intervention was assessed by placing an electrode at a specific location and monitoring changes in pO2 [17]. We have now performed such experiments with a micro
electrode and found a range of baseline pO\textsubscript{2} values and response to respiratory challenge similar to those seen using MRI.

We have now shown both that there is distinct intra tumoral heterogeneity in baseline oxygenation in the Dunning prostate AT1 tumor and also in the response to intervention. In common with our previous observations a three fold change in FO\textsubscript{2} seems to lead to a threefold response in tumor pO\textsubscript{2}. However, the rate of change in highly variable. Preliminary data with 8 min time resolution suggest that well oxygenated regions respond rapidly, whereas those poorly oxygenated require much longer. Such observations could have significant implications for patient inhalation times prior to therapy: while previous work had shown that Pre Irradiation Breathing Times (PIBT) could substantially influence the effect of oxygen or carbogen breathing [18], the differential response of individual tumor regions may not have been fully appreciated.

In developing a new technique it is important demonstrate its reliability, robustness and general application. We and several other groups have now applied the FREDOM approach to tumor oximetry. Initially investigators favored intra venous or intra peritoneal administration of emulsions of fluorocarbons. While material became trapped in tumors and could be used to report pO\textsubscript{2} [19-22], it became increasingly apparent that material delivered via the vasculature tended to bias measurements towards well perfused tumor regions [22]. Indeed, recent measurements by Griffiths et al. have confirmed such a bias [23]. Furthermore, the use of emulsions to carry the PFCs tend to lead to extensive uptake by the reticuloendothelial system with hepatomegaly. Intra tumoral administration is minimally invasive provided that a fine sharp needle is applied, as we have used here. We have now extended our work from the Dunning prostate R3327-AT1 tumor, which is poorly differentiated, has only microscopic necrotic foci and is firm, to the 13762 breast tumor, which has less structure and considerable cystic fluid. Here, we have simply reported the ability to measure dynamic changes in the breast tumor oxygenation, but in the accompanying work (Song et al., this volume), we show more extensive results.
Since the MR and electrode approaches appear to give similar results one may debate the relative their merits. Clearly, MR is very expensive, with a typical imaging system costing upwards of $1 M, compared with $60 000 for the Eppendorf and < $5 000 for a laboratory micro electrode system. However, MR facilitates the simultaneous measurements of dynamic changes in response to intervention at multiple points within a tumor. While we were able to follow changes in pO₂ at specific regions using a needle electrode with placement at sequential locations accompanied by cycling of the intervention, such an approach would be less satisfactory for other interventions, and even here, may have led to some conditioning or hysteresis. The FREDOM approach may be readily combined with other measurements such as blood flow/perfusion [24], pH [25] or metal ions by infusion of appropriate reporter molecules [26].

As a reporter molecule HFB has many advantageous properties. It is cheap, readily available, and exhibits minimal acute toxicity \( (LD_{50} > 25 \text{ g/kg}) \) [27]. No signs of renal or hepatic toxicity have been found [28] and others have tested doses as high as 50 g/kg (twice weekly) orally in rats over 35 weeks [29]. We typically find substantial clearance from tumors within 24 h, though this does limit our measurements to acute response to interventions [12]. High symmetry within the molecule leads to a single \(^{19}\text{F} \) MR resonance providing optimal SNR. The \( R_1 (=1/T_1) \) is highly sensitive to pO₂ while showing little response to temperature [9]. Long \( T_1 \)s up to 14 s appear to make HFB less efficient for spin lattice relaxometry, but use of the pulse burst saturation recovery approach minimizes the length of the experiment [10] and a large range of \( T_1 \) values is a requisite for sensitivity to changes in pO₂. The long transverse relaxation time (\( T_2 \)) is ideally suited to echo planar imaging.

The ultimate value of a novel technique will depend on its adoption by multiple laboratories, and the significance of the results that can be generated. We believe that the FREDOM approach is versatile and we are demonstrating increasing applications, and thus, we foresee expanded future application of the technique.
Reference:


**Figure legends**

**Figure 1**
Comparison of oxygenation in size-matched groups of AT1 tumors based on $^{19}$F MR EPI relaxometry (left) and electrode polarography (right), when rats inhaled 33% O$_2$. Small tumors are shown at top (volume < 2 cm$^3$) and large tumors below (volume > 3.5 cm$^3$). Each method shows a significant difference in tumor oxygenation for small versus large tumors (p< 0.0001).

**Figure 2**

a) Dynamic changes in pO$_2$ of six specific regions of an AT1 tumor. The three high pO$_2$ regions had significantly different pO$_2$ (* p< 0.05) from those with low pO$_2$ at each time point. Within 8 mins of elevating inspired O$_2$ the three high pO$_2$ voxels had significantly increased pO$_2$ (p< 0.05) while the low pO$_2$ voxels required > 24 mins to show significant changes. All six regions were observed simultaneously using the FREDOM approach.

b) Dynamic changes in pO$_2$ of six specific regions of an AT1 tumor. The electrode was placed in one location at a time and inhaled gases cycled for subsequent locations.
Figure 1
Regional Tumor Oxygen Tension and Blood Flow: Correlation Studies Using $^{19}$F PBSR-EPI of Hexafluorobenzene

Y. Song, R. P. Mason, S. Hunjan, A. Constantinescu, E. W. Hahn, and P. P. Antich, Department of Radiology, UT Southwestern Medical Center, Dallas, Texas, USA

Introduction: It is recognized that therapeutic efficacy may be influenced by tumor oxygenation. In particular, hypoxic tumors resist radiotherapy. We have recently shown the feasibility of monitoring tumor oxygen tension based on $^{19}$F PBSR-EPI of hexafluorobenzene (HFB) [1]. We also found that HFB clears from tumors over a period of hours [2]. Since HFB is a non-ionic freely diffusible tracer, it appeared that clearance rate would provide an indication of relative tumor blood flow (TBF). We have now investigated the feasibility of mapping the clearance rate of HFB and correlating this putative blood flow marker with corresponding $pO_2$.

Methods: Dunning prostate R3327-AT1 or breast 13762 NF adenocarcinoma was implanted in a skin pedicle on the forehead of a rat. When the tumor reached 1–2 cm diameter, 40 µl HFB were injected directly into the tumor (IT), both centrally and peripherally. The rat was maintained under general gaseous anesthesia (33% O$_2$, 66% N$_2$O and 0.5% methoxyflurane). A homebuilt tunable 2 cm $^1$H/$^{19}$F single turn solenoid coil was placed around the tumor and MR experiments were performed using a 4.7 T magnet equipped with actively shielded gradients. 3D $^1$H images were acquired for anatomical reference and corresponding $^{19}$F images were obtained to show the distribution of HFB. Tumor oxygenation was assessed using $^{19}$F PBSR-EPI of HFB. By applying the acquisition protocol ARDVARC (Alternated Relaxation Delays with Variable Acquisitions to Reduce Clearance effects) [2], we achieved $R_1$ maps in 8 min. A series of maps were acquired over a period of 2 hours with respect to respiratory changes. $pO_2$ maps were then generated by applying the relationship: $pO_2$(torr) = [$R_1$(s$^{-1}$) - 0.074]/0.0016 to the $R_1$ maps. The data also allowed us to produce a clearance map based on EPI images with the longest delay (90 s).

Results: $pO_2$ maps were generated with a typical precision of 2 – 5 torr and 30 – 100 individual voxels within a tumor. For many regions, the HFB signal intensity was found to decline exponentially with a typical clearance half-life ranging from $T_{1/2}$ = 700 to 1200 min, though many voxels indicated no apparent changes.

Discussion: Regional tumor oxygen tension and blood flow are important physiological parameters and the opportunity to measure both simultaneously would be of value in physiological research. Based on the preliminary data presented here, we believe that clearance of HFB provides an indication of relative TBF by analogy with studies of cerebral blood flow using freon-23 [3]. In future studies, such measurements will be rigorously evaluated.

References:

This study was supported in part by grants from The American Cancer Society (RPM), The Whitaker Foundation (RPM), the DOD Breast Cancer Initiative (YS), and NIH BRTP.
TUMOR OXYGEN DYNAMICS: COMPARISON BETWEEN $^{19}$F MR-EPI OF HEXAFLUOROBENZENE AND FREQUENCY DOMAIN NIR SPECTROSCOPY

Song$^{1,2}$, Y.; Worden$^{1}$, K. L.; Jiang$^{1}$, X.; Zhao$^{2}$, D.; Constantinescu$^{2}$, A.; Liu$^{1}$, H.; and Mason$^{2}$, R. P.

$^{1}$Joint Graduate Program in Biomedical Engineering, $^{2}$Department of Radiology, UT Southwestern Medical Center, Dallas, TX 75235, USA

Introduction: Oxygen plays a key role in tumor therapy and may be related to tumor development: e.g., angiogenesis and metastasis. Using noninvasive techniques to accurately measure oxygenation could assist in developing novel therapies. Here, we have used $^{19}$F MR-EPI relaxometry of hexafluorobenzene (HFB)$^{[1]}$ to monitor tissue oxygen tension ($pO_2$) of rat breast tumors and compared the results with changes in hemoglobin saturation (sO$_2$) and concentration in the vasculature of the tumors observed using a new dual wavelength homodyne near-infrared (NIR) system.

Methods: Breast 13762 NF adenocarcinomas were implanted in skin pedicles on the forebacks of adult female Fischer rats. Once the tumors reached ~1 cm diameter, the tumor blood sO$_2$ was assessed by NIR spectroscopy using a dual wavelength NIR system (758 nm and 782 nm) in transmission geometry$^{[2]}$. The tumor blood volume and sO$_2$ were calculated from the light amplitude. The rats were maintained under general gaseous anesthesia (33% O$_2$, 66% N$_2$O and 0.5% methoxyflurane). Once stable baseline measurements were achieved, the inhaled gas was altered to pure oxygen or carbogen and dynamic changes were observed over a period of two hours. Both the magnitude and rate of change of sO$_2$ were examined. Following the NIR experiments, 40 μl HFB were injected directly into both central and peripheral regions of the tumors. A tunable 2 cm $^1$H/$^{19}$F single turn solenoid coil was placed around the tumor and MR experiments were performed using a 4.7 T magnet. Regional tumor $pO_2$ was estimated using the relationship: $pO_2$ (torr) = [R1 - 0.074]/0.0016, where R1 is the spin lattice relaxation rate of HFB. Twenty-three $pO_2$ maps were produced in 3 hours with respect to respiratory challenge.

Results: NIR showed significant changes in vascular oxygenation accompanying respiratory interventions. $^{19}$F MR-EPI also showed significant changes in tissue $pO_2$, with considerable regional heterogeneity in both absolute values and rate of change accompanying interventions. Generally, changes in vascular sO$_2$ preceded tissue $pO_2$, particularly for smaller tumors.

Discussion: Regional tumor $pO_2$ and blood sO$_2$ are important physiological parameters. The capability to measure them will provide insight into progressive physiological changes in a tumor accompanying interventions. NIR has the advantage of being entirely noninvasive, but the MRI approach clearly reveals detailed oxygenation heterogeneity. We believe that better understanding and monitoring of tumor oxygenation can lead to improved tumor therapy.

References:

Acknowledgments:
Supported in part by grants from The American Cancer Society (RPM), The Whitaker Foundation (RPM and HL), the DOD Breast Cancer Initiative (YS), and NIH BRTP.
Molecular Determinants of Sensitivity to Anti-tumor Agents

Tumor oxygenation and measurement of regional dynamic changes
Ralph P. Mason, Sandeep Hunjan, Ana Constantinescu, Yulin Song, Eric W. Hahn, and Peter P. Antich, Advanced Radiological Sciences, U.T. Southwestern Medical Center, Dallas, Texas and Christian Blum and Peter Peschke, Deutsches Krebsforschungszentrum, Heidelberg, Germany

Therapeutic efficacy may be influenced by tumor oxygenation. In particular, hypoxic tumors resist radiotherapy and may be good candidates for hypoxia selective cytotoxic agents. We recently described a novel approach to measuring regional tumor oxygen tension using $^{19}$F pulse burst saturation recovery (PBSR) nuclear magnetic resonance (NMR) echo planar imaging (EPI) relaxometry of hexafluorobenzene (HFB) (1). We have now compared oxygen tension measurements in a group of size matched Dunning prostate rat tumors R3327-AT1 made using this new method with a traditional polarographic method: the Eppendorf Histograph. We also demonstrate extension of the MR techniques to rat breast tumors.

Methods: Rat Dunning prostate R3327-AT1 or breast 13762 NF adenocarcinomas were examined at a volume <2 cm$^3$ or > 3.5 cm$^3$: for MRI 40 μl HFB were injected directly into the tumor, both centrally and peripherally. The rat was maintained under general gaseous anesthesia (33% O$_2$, 66% N$_2$O and 0.5% methoxyfluorane). A tunable 2 cm $^1$H/$^{19}$F single turn solenoid coil was placed around the tumor and MR experiments were performed using a 4.7 T magnet equipped with actively shielded gradients. Tumor oxygenation was assessed using $^{19}$F PBSR-EPI of HFB. By applying the acquisition protocol ARDVARC (Alternated Relaxation Delays with Variable Acquisitions to Reduce Clearance effects), we achieved R1 maps in 8 min. A series of maps was acquired over a period of 2 hours with respect to respiratory challenges. pO$_2$ maps were then generated by applying the relationship: pO$_2$ (torr) = (R1 -0.074)/0.0016. In parallel experiments pO$_2$ was determined polarographically

Results: Similar oxygen tension distributions were found using $^{19}$F MRI or polarography and both techniques showed that tumors with volume > 3.5 cm$^3$ were significantly (p < 0.0001) less well oxygenated than smaller tumors (volume < 2 cm$^3$). Using the $^{19}$F EPI approach we also examined response to respiratory challenge. Increasing the concentration of inspired oxygen from 33% to 100% O$_2$ produced a significant increase (p < 0.0001) in tumor oxygenation for a group of small tumors. In contrast no change was observed in the mean pO$_2$ for a group of large tumors. Consideration of individual tumor regions, irrespective of tumor size showed a strong correlation between the maximum pO$_2$ observed when breathing 100% O$_2$, as compared with mean baseline pO$_2$.

Conclusions: These results further demonstrate the usefulness of $^{19}$F EPI to assess changes in regional tumor oxygenation. The ability to measure pO$_2$ could be valuable in pre-clinical evaluation of novel therapies and ultimately allow therapy to be individualized and optimized for patients.

This work was supported in part by The American Cancer Society (RPM), The Whitaker Foundation (RPM), DOD Breast Cancer Initiative (YS), Verein zur Förderung der Krebskennung und Krebsablauf e.V. Heidelberg (PP) and the NIH BRTP Facility #5-P41-RR02584.