Magnetic resonance imaging contrast agents for chemical sensing

by

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Abstract

Magnetic resonance imaging (MRI) is frequently used for examining the human body. MRI contrast agents currently used in the clinic assist physicians in locating problematic areas, but other tools are needed to interrogate the chemical environment of these areas once found. The fast clearance rate of contrast agents, in particular, makes quantitative assessment of local tissue with MR difficult. This thesis focuses on the development of solid MRI contrast agents designed for long-term, quantitative monitoring of local metabolites.

Two types of contrast agents are described in this thesis. Contrast agents sensitive to oxygen were made by encapsulating low molecular weight siloxanes in a polydimethylsiloxane (PDMS) matrix. The PDMS matrix is biocompatible and prevents clearance of the contrast material. The longitudinal relaxation time, \( T_1 \), of these agents is proportional to the amount of dissolved oxygen in the material and is indicative of local oxygen tension. A microparticulate formulation of these agents was tested in a rat model and was shown to be stable for up to one month \( \textit{in vivo} \). These results suggested that the encapsulated agents enjoyed an order of magnitude improvement in stability over a simple liquid contrast agent injection.

Contrast agents sensitive to changes in pH were made of a polymerized hydroxethylmethacrylate (HEMA) hydrogel and used with the microresonator sensor previously developed in the Cima Lab. These sensors reversibly detected changes in pH in flowing liquid for up to three weeks, and detected the different pH of a tumor compared to control locations.

Thesis Supervisor: Michael J. Cima
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Contents

Chapter 1 .............................................................................................................................. 19
  1.1 Sensors in clinical medicine ................................................................. 19
  1.2 Clinical need for local monitoring .................................................... 20
  1.3 Medical Imaging .................................................................................. 20
  1.4 Magnetic Resonance phenomenon .................................................... 21
     1.4.1 MR Relaxation ........................................................................... 22
     1.4.2 Measuring $T_1$ and $T_2$ .......................................................... 24
  1.5 Image Contrast in MRI ........................................................................ 27
     1.5.1 Contrast Agents ....................................................................... 29
  1.6 Summary of Chapters .......................................................................... 31
 References ................................................................................................................... 33

Chapter 2 .............................................................................................................................. 35
  2.1 Motivation .............................................................................................. 35
  2.2 Current Technologies ............................................................................ 38
  2.3 Magnetic Resonance in Oxygen Sensing ........................................... 40
  2.4 Siloxanes as oxygen contrast agent ..................................................... 41
  2.5 Conclusions ........................................................................................... 47
 References ................................................................................................................... 48

Chapter 3 .............................................................................................................................. 53
  3.1 Theory ...................................................................................................... 53
  3.2 Experiments ............................................................................................ 58
  3.3 Components of sensor response ......................................................... 59
     3.3.1 Intrinsic Relaxation time ............................................................. 60
     3.3.2 Oxygen contribution to relaxation time .................................... 63
     3.3.3 Transverse relaxation time ......................................................... 68
  3.4 Optimization strategy ............................................................................ 68
  3.5 Conclusion .............................................................................................. 70
 References ................................................................................................................... 71

Chapter 4 .............................................................................................................................. 74
  4.1 Motivation .............................................................................................. 74
  4.2 Siloxane/PDMS Oxygen sensors ......................................................... 76
  4.3 MRI Studies ............................................................................................ 83
  4.4 DDMPS/PDMS Microparticles ............................................................. 95
  4.5 Conclusion .............................................................................................. 98
 References ................................................................................................................... 101

Chapter 5 .............................................................................................................................. 104
  5.1 Signal isolation in the MRI ................................................................. 104
  5.2 Inspired gas experiments ................................................................. 106
5.3 Circulation Restriction Experiments .......................................................... 112
5.4 Data Analysis ......................................................................................... 114
5.5 Conclusions .......................................................................................... 115
5.6 Materials and Methods ........................................................................ 115
References .................................................................................................... 118

Chapter 6 .......................................................................................................... 120
6.1 Motivation .............................................................................................. 120
6.2 Existing technologies ............................................................................. 121
6.3 MR Technologies .................................................................................. 122
6.4 PolyHEMA as a pH-sensitive material .................................................. 125
6.5 pH measurements using micro-resonator device .................................... 131
6.6 Tumor Model ......................................................................................... 134
6.7 Conclusion .............................................................................................. 139
References .................................................................................................... 140
List of Figures

Chapter 1

Figure 1.1: The three main steps involved in magnetic resonance imaging – spin alignment, excitation, and relaxation, are illustrated in this figure. Note that while aqueous samples are the most common sample type encountered in clinical situations, any nuclei resonating at the proper frequency can be measured. .......................... 22

Figure 1.2: Schematic showing T<sub>1</sub> data acquisition. The magnetization is first inverted to the negative z-axis, where the varying wait time allowed for different amounts of relaxation recovery. Several example data points are shown on the right hand side of the figure. Magnetizations at different points of recovery (upper right hand corner) are color-coordinated with points on the plot (lower right hand corner) to show how the acquired data relates to the relaxation curve. A larger number of data points are acquired near the beginning of the recovery process to ensure accurate tracking of the recovery curve. .......................................................... 25

Figure 1.3: The spin-echo sequence is a method used to eliminate equipment errors that prevent accurate T<sub>2</sub> determination. The spin-echo refocuses the spins by inverting the magnetization vectors and causing phase accrual in the opposite direction. The example above shows the refocusing pulse being applied at a time of TE/2 after excitation, and data is acquired at TE/2 after application of the refocusing pulse. If a constant amount of phase accrual happened in each time period, they would be equal and opposite, thus allowing them to cancer each other. ......................... 26

Figure 1.4: T<sub>1</sub>-weighted images of the brain and the corresponding signal recovery curves. The signal at any particular TR would result in different image intensities. Reference: McRobbie, et al. MRI from Picture to Proton, 2nd ed., p. 34.............. 28

Figure 1.5: T<sub>2</sub>- weighted images of the brain acquired with a spin-echo sequence and T<sub>2</sub> decay curves. It can be seen that free liquids have a higher intensity at long TE. Reference: McRobbie, et al. MRI from Picture to Proton, 2nd ed., p. 36.............. 28

Figure 1.6: The effect of T<sub>1</sub> contrast agents can be seen by comparing the images before (left) and after (right) contrast administration. The boundary of the tumor on the left side of images is highlighted against tissue background with Gd-based contrast. Reference: McRobbie, et al. MRI from Picture to Proton, 2nd ed., p. 43............. 30

Figure 1.7: The SPIO nanoparticles used in this patient results in a decrease in intensity of normal tissue, allowing a tumor to be visualized. Reference: McRobbie, et al. MRI from Picture to Proton, 2nd ed., p. 44................................. 30
Chapter 2

Figure 2.1: A higher dose of radiation is required to overcome tumor hypoxia in patients with prostate cancer. Specifically, 165 Gy of radiation is required to achieve the same therapeutic outcome compared to 140 Gy in non-hypoxic tumors. Reference: J. Wang, et al., Br J Radiology (2006) 79, p905 .............................................................. 37

Figure 2.2: The pi orbital of oxygen molecules contain two unpaired electrons, making the molecule paramagnetic. Reference: Burrows, A. et al., Chemistry 3, 2nd ed. .... 42

Figure 2.3: Structure of linear siloxanes studied in this chapter. The siloxanes are all commercially available and were purchased from Sigma-Aldrich (USA). Abbreviations for the siloxanes are as follows: Hexamethyldisiloxane (HMDSO), Octamethyltrisiloxane (OMTSO), Decamethyltetrasiloxane (DMTSO), Dodecamethylpentasiloxane (DDMPS) .......................................................... 44

Figure 2.4: The measured longitudinal relaxation times of siloxanes (top) equilibrate to changes in oxygen partial pressure (bottom) within 10 minutes. The response is completely reversible after multiple cycles of gas switches. ................................. 45

Figure 2.5: The change in the $T_1$ of siloxanes is greatest at low oxygen partial pressures and corresponds to the oxygen concentrations typically encountered in the body. 46

Figure 2.6: The longitudinal relaxation rate is linear with oxygen partial pressure. Each data point represents an average of 6 measurements and the measurements were performed at 40°C ................................................................. 47

Chapter 3

Figure 3.1: SNR vs $T_1$, mat. This plots shows the relationship between calculated SNR and the intrinsic $T_1$. DDMPS, DMOO, and DI water have different oxygen contributions. This difference is highlighted by an increase in SNR from DI water to DDMPS. The dotted line represents the maximal SNR possible at a particular relaxation time that is achieved when the contribution of oxygen brings the relaxation time to 0. Relaxation times were obtained on a benchtop NMR relaxometer............................................................. 61

Figure 3.2: The longitudinal and transverse relaxation time of molecules as a function of correlation time. Figure reproduced from Bloembergen, E.M. Purcell, R.V. Pound "Relaxation Effects in Nuclear Magnetic Resonance Absorption" Physical Review 1948, 73, 679-746 .......................................................... 61

Figure 3.3: Correlation between oxygen solubility and diffusivity of solid LMW siloxanes in PDMS at 70% w/w and liquid siloxanes. The oxygen solubility of matrix embedded siloxanes were scaled to compensate for effectively smaller volume. Both neat and matrix-embedded siloxanes respond linearly as predicted by
the oxygen response equation. Relaxation times were measured with MRI with an
echo time of 100 ms. ................................................................. 65

Figure 3.4: Linear fit of oxygen solubility of solvents and oxygen contribution to the
relaxivity. Polarity strongly predicts oxygen solubility as can be seen in the plot.
The oxygen solubility of hexane and DI water, for example, varied by an order of
magnitude with DMSO in between the two. The cluster of siloxane samples on the
right side of the plot illustrates that samples with different viscosity plays a more
significant role when comparing materials with similar oxygen solubility.
Relaxation times were obtained a benchtop NMR relaxometer..........................66

Figure 3.5: The oxygen contribution to the relaxivity, $R_{1,ox}$, plotted against the oxygen
solubility / total diffusivity. The different material groups can be seen to follow the
trend of increasing oxygen contribution with a larger product of oxygen solubility or
lower oxygen diffusivity. Relaxation times were measured on a benchtop NMR
relaxometer................................................................. 67

Chapter 4

Figure 4.1: Structure and chemical formula for PDMS. The chemical structure of Sylgard
184 is not fully disclosed by Dow Corning, but it is modified to include a vinyl
group for crosslinking purposes................................................. 77

Figure 4.2: PDMS/Siloxane devices can be made in different sizes and be tailored for
specific anatomical dimensions. Left: Devices measuring 1mm to 4mm were made
by molding. Right: A device measuring 1.2mm loaded in a 16 Gage needle.........78

Figure 4.3: Both the longitudinal relaxation time and the change in relaxation time are
proportional to the amount of DDMPS embedded in the PDMS matrix. The
response was reversible and equilibrates within 10 minutes of gas change.........79

Figure 4.4: DDMPS does not partition into either deionized (DI) water or fetal bovine
serum (FBS). DDMPS can, however, freely diffuse in and out of the sensor, as
evidenced by the weight loss experienced by sensors placed in air. The lack of
weight loss for sensors placed in an aqueous environment can be attributed to the
low solubility of siloxanes in those solutions. Thus, the aqueous solution creates a
barrier for transport of low molecular weight siloxanes into the headspace of the
container. The favorable partition coefficient is a likely factor that keeps the
materials functionally stable in the body........................................81

Figure 4.5: DDMPS/PDMS Devices autoclaved while immersed in water retains its
performance characteristics compared to devices autoclaved in air. The aqueous
environment prevents siloxane escape by creating a barrier that prevents transport of
siloxanes into the headspace of the container..................................................82
Figure 4.6: MRI scan of 4 different siloxanes embedded in PDMS at 70% weight/weight. The siloxanes were contained in glass vials, which are placed in a bottle with DI water to minimize susceptibility artifacts. The temperature was kept constant at 37°C with the use of a forced hot air circulator. A fast spin-echo pulse sequence with inversion recovery was used to generate the images at 8 different inversion times; intensity in each of the image can then be used for extracting the T1 relaxation time.

Figure 4.7: A representative T1 relaxation curve of the siloxane samples as fitted to the inversion recovery equation. The relevant regions of interest (ROI) were first selected and the intensity within the ROI averaged at each inversion point. A total of 8 inversion points were used for fitting each sample to the model.

Figure 4.8: 70% DDMPS/PDMS devices exhibit double-exponential relaxation behavior when the data is acquired at low TE (0.5ms). The data contained signal from both DDMPS and PDMS, and fitting to the appropriate model is required to extract the correct relaxation times.

Figure 4.9: MRI scans of phantoms containing different siloxanes. The phantoms were in glass tubes and are immersed in a container filled with DI water to minimize magnetic susceptibility differences. PDMS has a T2 of 20 ms at 7T, and the average intensity of PDMS phantom drops to less than 20% of its initial value at an echo time of 100ms. Coupled with a lower initial intensity value, signal from PDMS would constitute less than 1% of acquired signal at 100ms. 70% DDMPS 30% PDMS phantoms have lower intensity values throughout the measurement period due to an effectively smaller amount of sample but the extracted T2 value is not significantly different from pure DDMPS.

Figure 4.10: Normalized relaxation time of DDMPS and 70%DDMPS measured using the MRI at an echo time (TE) of 100ms. The averaged relaxation times for DDMPS and 70% DDMPS are 2.6±0.06s and 2.55±0.12s, respectively. The longitudinal relaxation time of DDMPS (solvent) dominates the overall signal at high echo times. Additionally, the relaxation data is well fitted by a mono-exponential fitting scheme.

Figure 4.11: Comparison between neat siloxane and siloxane incorporated into PDMS at 70w/w% in air (n = 3). The relaxation rates of neat siloxane material are not significantly different from material incorporated into PDMS, indicating that signal from the PDMS matrix can be effectively suppressed. The data points are based on 6 measurements in the MRI at 37°C and error bars represent standard deviations of measurements.

Figure 4.12: Evolution of Sylgard 184 PDMS relaxation times as curing occurs. The T1 of PDMS decreased by around 13% (from 550ms to 480ms) while the T2 decreased by 78% (from 114ms to 25ms) as the polymer turns from liquid into solid.
Figure 4.13: Kinetics of response of 70% DDMPS 30%PDMS phantoms are similar to liquid DDMPS. These phantoms were exposed to changing oxygen partial pressure in the MRI, and image intensity was used as a surrogate indicator of T1. Images were taken every 2 minutes with a coronal orientation and the intensity in the phantoms was averaged to estimate time to steady state. We found that steady state was achieved in less than 10 minutes for phantoms of all sizes. ........................................ 92

Figure 4.14: Relaxation rates of 70% DDMPS/PDMS phantoms were measured in the MRI at 3 different temperatures (n=3). Error bars denote standard deviations. The measured relaxation rate was found to have a negative correlation with temperature at all oxygen partial pressures. ................................................................. 94

Figure 4.15: The microparticles are synthesized in a size range that would enable their retention in most tissue types. Once the particle suspension is injected, the filtering of saline into the surrounding tissue allows the particles to come into contact with each other. The non-specific interaction of PDMS allows the particles to coalesce into a single depot sensor. .......................................................... 96

Figure 4.16: An explanted piece of rat muscle tissue with injected microparticles shows the fusion of particles in the body. The particles resided inside the animal for 4 weeks and resisted displacement from normal animal movement......................... 97

Figure 4.17: The impact of process parameters on particle size. One of the process parameters is varied on each row while the rest were held to be the same. The particles were cured using the same method and the washing steps were the same across different samples. ................................................................. 99

Figure 4.18: The longitudinal relaxation times of the microparticles are similar to the relaxation times measured with larger devices. ...................................................... 100

Chapter 5

Figure 5.1: Tissue background signal can be suppressed with the use of long echo times in a spin-echo sequence. The intensity of muscle signal can be seen to significantly decrease when the echo time was increased from 10ms (left) to 100ms (right). Both the implanted device (top) and injected microparticles (bottom) retained a relatively high intensity even at a high TE................................................................. 106

Figure 5.2: Oxygen measurements extracted from sensors immediately after injection (top) and 4 weeks after injection (bottom) are plotted against pulse oximeter measurements (n = 4). Each data point represents an average of 6 measurements in each animal and error bars denote standard deviations of measurements. A linear regression line is used to find the correlation between sensor measurements and pulse oximeter readings. The values of the regression slopes are found to be similar in both measurements, indicating similar correlation between pulse oximeter readings and sensor measurements over the 4-week period. Deviations from the line
are likely due to variations in local tissue oxygen level and differences in injection location. Blue: oxygen (initial); red: air; cyan: oxygen (reverse) ..................... 110

Figure 5.3: Maps of extracted oxygen tension can be overlaid on anatomical images to provide both physical and biochemical information. These two representative MRI images are coronal slices of the leg and are taken from animals breathing oxygen (left) and air (right) respectively. ................................................................. 111

Figure 5.4: H&E stained micrographs showed that the 70% DDMPS/PDMS microparticles fused into a depot that resisted migration after injection. There is minimal foreign body reaction to the injected microparticles as evidenced by the thin fibrous capsule surrounding the injection site......................................................... 111

Figure 5.5: The injected contrast agents resisted physical stress and continued functioning irrespective of blood flow. Oxygen tension extracted during circulation restriction experiment (n=3). Error bars denote standard deviations across different animals. Pulse oximeter readings become unavailable when the pressured was raised to 75 mmHg and 150 mmHg pressure levels and are not presented here. The oxygen level was observed to return to the initial level after the pressure cuff was released. Representative pixel maps of extracted oxygen tension are overlaid with MRI scans of the animal at different cuff pressure levels. The pictures are coronal slices of the animal’s leg. Sensor migration, even at the highest pressure level applied, was not observed. ........................................................................................................ 113

Figure 5.6: After signal from the matrix material and muscle tissue background are suppressed with appropriately chosen pulse sequence parameters, unwanted signal is excluded with proper ROI selection. An automated script then calculates the relaxation times and extracts the metabolite concentrations........................................ 115

Chapter 6

Figure 6.1: Extracellular, intratumoural pH is depressed from metabolic changes as a result of the irregular vasculature network found in a tumor. Tumors responding to a chemotherapeutic were found to have a pH value further depressed to pH 5.5, whereas the pH in tumors resistant to the chemotherapeutic remains unchanged. Reference: D. Linder and D. Raghavan. Br J Cancer (2009), 100, p1287............. 121

Figure 6.2: Schematic representations of the distribution of spins, aligned with and against the field (upper and lower energy levels, respectively) (above) and simulated NMR spectra (below) for two chemically distinct pools of nuclei (left), two spins after a saturation pulse has been applied to one pool (middle), and for a system undergoing chemical exchange after a saturation pulse has been applied to one pool (night). Figure and caption reproduced from Sherry and Woods11.............................. 123

Figure 6.3: The transverse relaxation time of hydrogels and biomolecules with specific functional groups change with pH. These materials are used as a starting point for

**Figure 6.4:** The transverse relaxation rate increase with increasing concentration of HEMA, and the response to changing pH values also increases with the concentration of HEMA in the reaction mixture. These effects are due to increased concentration of active CEST sites on the polymer and changes in the physical structure of the polymer.

**Figure 6.5:** The transverse relaxation times of HEMA-BIS hydrogels are determined by both the concentration of HEMA and concentration of BIS in the reaction mixture. The relaxation times observed in the more highly crosslinked polymer (top) are lower at all pH values compared to similar polymers (bottom) with the same HEMA content. The overall shape of pH response, however, remained similar at different crosslinking levels.

**Figure 6.6:** The polyethylene shell enclosing the HEMA-BIS gel ensures that proton transport into the gel can only occur from the opening at the top. These devices were used to evaluate the kinetics of change of the pH gels.

**Figure 6.7:** The measured transverse relaxation time of the pH gels equilibrate within 2 hours of a step change in pH. The pH was alternated between 7.4 and 5.5 in this experiment, and the results were measured on the NMR MOUSE setup. Each data point represented the averaged result of 3 devices.

**Figure 6.8:** This electron micrograph of HEMA-BIS gels reveals the macroscopic pores present in the gel. Fast transport of solutes and analytes into and out of the gel is possible with these pores, which present a large diffusion area and fast kinetics for the sensors.

**Figure 6.9:** The microresonator device measures roughly 1.5mm in diameter and can be injected through the bore of a biopsy needle. The sensors are coupled to an external reader that allows for MR excitation and measurements.

**Figure 6.10:** The HEMA-BIS gel detected changes in pH in a microresonator device for 3 weeks. The devices was placed in a tube where pH-adjusted saline from a temperature controlled bath flows through. The bath temperature and pH were monitored using an external pH probe.

**Figure 6.11:** The response of HEMA-BIS gel can be modeled similarly to a weak acid.

**Figure 6.12:** Devices implanted inside the tumor showed a lower transverse relaxation time compared to devices implanted near the tumor or at the contralateral flank.
Figure 6.13: H&E stained histographs of animals that received Doxorubicin (right) are virtually indistinguishable from animals that received saline (left) injections. The extensive necrotic regions (blanks and ligh pinks areas) seen in both sets of pictures produced the confounding results described.

Figure 6.14: Measured transverse relaxation time of sensors implanted in mouse tumors. Red lines denote animals that received daily Doxorubicin IP injections and black lines denote animals that received daily saline IP injections.

Figure 6.15: The difference between relaxation time measured on the first day (pre-dosing) and on the day of animal sacrifice. The results did not show statistical significance between animals dosed with drug and animals dosed with saline.
List of Tables

Chapter 3

Table 3.1: Relaxation times of materials measured at 1 atm oxygen and deoxygenated state. * denotes transverse relaxation times that are substantially similar to longitudinal relaxation times. T1, mat correlates inversely with molecular weight both within a family of materials and between different material systems. Siloxanes with ring structures have a lower intrinsic relaxation time compared to linear siloxanes; the intrinsic relaxation time of OMCTS, for example, is about 40% lower than that of DMTSO even though their molecular weights are similar. .................. 63

Table 3.2: Physical parameters of materials tested......................................................... 67

Chapter 4

Table 4.1: The error introduced by temperature variations is comparable to measurement deviations. ........................................................................................................... 94
Chapter 1

Introduction

Up-to-date information is important for the proper functioning of many engineered systems, and is critical in the management of human diseases. One peek into an operating room would reveal arrays of sensors and monitoring equipment, all providing information on the patient so that timely action could be taken. Imaging is used to ascertain a diagnosis or to aid in adjusting the treatment regimen prior to many medical procedures. This thesis focuses on the use of contrast agents in medical imaging and combining imaging technology with sensing techniques. This chapter of the thesis will provide a background in magnetic resonance technology and a summary of the thesis.

1.1 Sensors in clinical medicine
Decision-making in evidence-based medicine relies on the ability to gather sufficient information about the patient and disease to make an accurate diagnosis. This information is gathered through both qualitative and quantitative methods. Qualitative information, such as family history and outward symptoms of the disease, is obtained from patient interviews. Quantitative information, on the other hand, is obtained through the use of medical sensors. These sensors could be as simple as a thermometer or as complex as a magnetic resonance imager. The primary application of sensors in medicine is to provide timely information so that interventions can be taken to correct for deviations from nominal values. Sensors can be further divided into those that sample systemic variables and those that perform local sensing based on the extent of sampling performed. These sensors, while both important, play very different roles in the management of diseases.
1.2 Clinical need for local monitoring

A blood draw is possibly the most common clinical technique for measuring systemic biomarkers. Systemic sampling is used to quantify the concentration of soluble markers and provides physicians with basic information about the body required to make an initial diagnosis. There are some conditions, however, that revolve around local disruptions of essential metabolic processes. These abnormal metabolic processes result in deviations in local pH and oxygen tension that cannot be detected with systemic sampling. Cancer is a prominent example with locally disrupted metabolic processes. Solid tumors are often characterized by a malformed vasculature network that result in depressed oxygen tension and chronic hypoxia in the tumor. Systemic measurements will not detect this abbreviation since the disruption to blood flow occurs only locally, and local monitoring is required. Another situation that would benefit from local monitoring is the detection of disease response in localized infections, such as Hepatitis C. Systemic detection of pathogen load is difficult because of the low viral concentration in the blood stream. A technology that accurately quantifies the concentration of Hepatitis C virus at the infection site (i.e. the liver) would enable a more precise determination of the disease end point and for the treatment to be tailored accordingly. A final example is the detection of cancer recurrence. The concentration of cancer biomarkers and related metabolic changes are often difficult to detect with systemic tests even with regular screening. A method that detects the local tumor marker level would allow cancer recurrence to be detected at an earlier stage.

1.3 Medical Imaging

The ability to peer into the human body has provided valuable insights to physicians on the physical basis of diseases. Imaging is, indeed, often used to
identify the causes of outward symptoms, and a recent survey of physicians has ranked medical scanners as one of the most important innovations of the last 25 years. Medical imaging technologies can be divided into devices that perform functional imaging, such as Positron Emission Tomography (PET), and anatomical imaging, such as Computed Tomography (CT). Anatomical imaging devices provide information on physical tissue structure, whereas functional imaging devices provide metabolic and chemical information in the tissue. Both types of information are often required for the diagnosis and management of diseases. Magnetic Resonance Imaging (MRI) is unique amongst imaging modalities where both anatomical and functional information are obtained within the same study. MRI, additionally, does not utilize ionizing radiation and is considered safe for longitudinal imaging. This could be an important consideration for monitoring of chronic conditions.

### 1.4 Magnetic Resonance phenomenon

Protons in atomic nuclei have positive charges and spins that make them behave like tiny magnets. When these spinning charges are placed in an external magnetic field, they align along the direction of the magnetic field and gives rise to a net magnetic moment that can be detected externally. This net magnetic moment is a vector that aligns either in the parallel or anti-parallel direction relative to the external magnetic field. This vector also precesses at a well-defined rate called the Lamour frequency. The Lamour frequency depends on the type of atom under examination and the magnetic field experienced by the atoms, and is given by the equation $\omega_0 = \gamma B_0$, where $\gamma$ is the gyromagnetic ratio and $B_0$ is the magnitude of the externally applied magnetic field. These spins, qualitatively, can be thought of as magnets precessing along the direction of the applied magnetic field (i.e. the z-direction). A radiofrequency (RF) pulse oscillating at the Lamour frequency can be used to manipulate the orientation of these precessing spins. The application of
a RF pulse of the appropriate magnitude and duration can induce a 90-degree rotation of the spins onto the x-y plane. The spins then relaxes back into alignment with the $B_0$ field according to a characteristic time constant. This phenomenon is illustrated in Figure 1.1.

![Figure 1.1](image)

Figure 1.1: The three main steps involved in magnetic resonance imaging – spin alignment, excitation, and relaxation, are illustrated in this figure. Note that while aqueous samples are the most common sample type encountered in clinical situations, any nuclei resonating at the proper frequency can be measured.

### 1.4.1 MR Relaxation

Magnetic relaxation is the event by which excited spins dissipate energy to return to equilibrium and is comprised of two simultaneous processes. The first process occurs as excited spins lose energy to other nuclei and electrons in the surrounding environment (collectively known as the lattice); this process is known as spin-lattice relaxation. Spin-lattice relaxation is induced by interaction with locally fluctuating magnetic fields. Each molecule in the lattice possessing a magnetic moment generates its own local field distortions with random thermal motion. These local field distortions act like local RF pulses that can re-orient the excited spins. Recall from the previous section that excitation of spins require a RF pulse at the Lamour frequency; spin-lattice relaxation similarly only occurs when these
local field distortions happen at the Lamour frequency. The relaxation time is therefore inversely proportional to the number of spins interacting at the Larmor frequency. The spin-lattice relaxation process is characterized by an exponential function given by the equation \( I = I_0(1 - 2e^{-\frac{t}{T_1}}) \).

A second process, known as spin-spin relaxation, describes a situation where spins lose energy without the energy being transferred to the lattice. This is commonly known as de-phasing and is observed as line broadening on a NMR spectrum or a reduction of signal magnitude in the time domain. The primary driver of spin-spin relaxation is local field gradients generated by tumbling molecules; instead of transfer of energy as in the case of spin-lattice relaxation, these field gradients cause a loss of phase coherence in the spin ensemble and thus a gradual loss of signal. Molecular motion is a major driver in spin-spin relaxation, and the process is characterized by the exponential function \( M_{xy} = M_0e^{-\frac{t}{T_2}} \).

The spin-lattice (\( T_1 \)) and spin-spin (\( T_2 \)) relaxation times of the same material can have remarkably different values despite both having a dependence on local field fluctuations. The reason behind this is that \( T_1 \) is only sensitive to fields fluctuating at the Lamour frequency whereas \( T_2 \) is affected by all random field fluctuations. These effects can be explained using the concept of correlational time as used in the equations below:

\[
\frac{1}{T_1} = \gamma^2 \frac{\tau_c}{1 + (2\pi v_0 \tau_c)^2} \quad \quad \frac{1}{T_2} = \gamma^2 \frac{(\tau_c + \frac{\tau_c}{1 + (2\pi v_0 \tau_c)^2})}{H^2}
\]
where $\gamma$ is the gyromagnetic ratio, $\overline{H^2}$ is the average of the local magnetic fields and thus representative of the strength of the dipolar interactions, $v_0$ is the Lamour frequency, and $\tau_c$ is the correlational time. The correlational time is dictated by factors related to molecular motion and has been extensively reviewed elsewhere\textsuperscript{2, 3}.

1.4.2 Measuring $T_1$ and $T_2$

A NMR pulse sequence is a series of radiofrequency (RF) pulses designed for spin excitation and data acquisition. NMR data is acquired when the spins are oriented perpendicularly to the externally applied main field so as to minimize interference from the main field. A typical pulse sequence first excites the nuclear spins into the x-y plane, and signal from the precessing spins is then recorded by a detector designed to record the emitted signal in the x-y plane. The evolving signal magnitude is tracked over time and used to extract the relaxation time constant with the appropriate model.

The inversion recovery (IR) sequence is the standard method for measuring $T_1$. Magnetization is first inverted 180 degrees to the antiparallel orientation by the exciting RF pulse. This is followed by a varying wait time, $\tau$, that allows the magnetization to recover from $-M_0$ towards $M_0$. Data is acquired after $\tau$ by exciting the magnetization to the x-y plane and then recording the intensity. The delay time $\tau$ essentially determines where the data point would be located on the $T_1$ curve and is increased for each subsequent measurement to acquire different points on the curve. The acquired intensity values can then be fitted with the equation $M = M_0(1 - 2e^{-\frac{\tau}{T_1}})$ (Figure 1.2).
Figure 1.2: Schematic showing $T_1$ data acquisition. The magnetization is first inverted to the negative $z$-axis, where the varying wait time allowed for different amounts of relaxation recovery. Several example data points are shown on the right hand side of the figure. Magnetizations at different points of recovery (upper right hand corner) are color-coordinated with points on the plot (lower right hand corner) to show how the acquired data relates to the relaxation curve. A larger number of data points are acquired near the beginning of the recovery process to ensure accurate tracking of the recovery curve.

The transverse relaxation time ($T_2$) is measured by tracking the evolution of signal intensity in the x-y plane. The detected signal represents the accumulated contribution from all nuclear spins in the volume of interest. $T_2$ relaxation is caused by a loss of phase coherence amongst the spins. This phase difference is caused by random fluctuations of the local magnetic field and the associated variations in precession frequency of different spins. The overall magnitude decreases when the magnitude of spins with incoherent phase is added. There are intrinsic and extrinsic causes for this loss of phase coherence. Intrinsic factors include spin interaction and the molecular environment experienced by the spins; these factors give rise to the characteristic $T_2$. Extrinsic factors, such as main field inhomogeneity, introduce additional phase incoherence independently of the
sample. This increase in phase accrual is not a true relaxation process, as it is not related to the material. It is, rather, caused by equipment imperfection and needs to be corrected for an accurate measurement of $T_2$.

This imperfection can be corrected through the use of spin-echoes. A spin-echo, generated through the application of a 180° refocusing pulse, inverts the phase differences that eventually allows phase coherence to be recovered. A refocusing pulse inverts the spins, resulting in phase accumulation in the opposition direction after the spins were excited to the x-y plane and phase accrual occurs. The signal is recorded at the echo time (TE), when the previously acquired phase difference is cancelled out and the phase coherence recovered (Figure 1.3).

![Diagram of spin-echo sequence]

**Figure 1.3:** The spin-echo sequence is a method used to eliminate equipment errors that prevent accurate $T_2$ determination. The spin-echo refocuses the spins by inverting the magnetization vectors and causing phase accrual in the opposite direction. The example above shows the refocusing pulse being applied at a time of TE/2 after excitation, and data is acquired at TE/2 after application of the refocusing pulse. If a constant amount of phase accrual happened in each time period, they would be equal and opposite, thus allowing them to cancel each other.
1.5 Image Contrast in MRI

Compared to other imaging technologies, MRI has a more complex contrast mechanism as both intrinsic and extrinsic factors affect the image contrast. The intrinsic factors are based on material properties such as the $T_1$ relaxation time. These can be manipulated through the use of contrast agents. Extrinsic factors are related to the choice of pulse sequence or pulse sequence parameters. These methods can be tuned to emphasize particular material properties, such as $T_1$ relaxation over $T_2$ relaxation. A combination of intrinsic and extrinsic factors determines the final image contrast.

$T_1$ contrast is generated with the use of short recovery (TR) times. Image intensity is directly proportional to the speed at which the signal recovers as dictated by its longitudinal relaxation time, $T_1$. Features with short $T_1$, when recorded at a short TR, would have a proportionally higher intensity than features with long $T_1$. Features with shorter $T_1$ are thus brighter in the images (Figure 1.4). $T_1$-weighted imaging has been used to selectively highlight tissue structures with short $T_1$ such as fat-based tissues over water-based tissues.

$T_2$ contrast is generated by manipulating the echo time (TE) in a spin-echo sequence. The echo time represents the time at which the signal is recorded in a spin-echo based pulse sequence. The intensity of features with short $T_2$ decays at a faster rate compared to features with a long $T_2$, and images recorded at long TE would emphasize this difference (Figure 1.5). $T$ image acquired using $T_2$ contrast would thus highlight structures with long $T_2$, such as fluid accumulation, compared to other tissue structures.
Figure 1.4: T₁-weighted images of the brain and the corresponding signal recovery curves. The signal at any particular TR would result in different image intensities. Reference: McRobbie, et al. MRI from Picture to Proton, 2nd ed., p. 34.

Figure 1.5: T₂-weighted images of the brain acquired with a spin-echo sequence and T₂ decay curves. It can be seen that free liquids have a higher intensity at long TE. Reference: McRobbie, et al. MRI from Picture to Proton, 2nd ed., p. 36.
1.5.1. Contrast Agents

The contrast between different features can be enhanced with MR contrast agents. These contrast agents are paramagnetic molecules with a large number of unpaired electrons. These unpaired electrons act collectively as a magnetic dipole that creates a large local field distortion. This field distortion increases the relaxation rates of surrounding molecules, which allows additional contrast to be generated using the pulse sequence manipulations described in the previous section.

T₁ contrast agents increase the intensity of pixels in the areas of interest. Clinical T₁ contrast agents are Gadolinium chelates that shorten the T₁ of surrounding molecules and allow difficult to visualize features to be seen (Figure 1.6).

T₂ contrast agents decrease the intensity of pixels in the areas of interest. Superparamagnetic Iron Oxide (SPIO) particles is a class of contrast agent used to lower the T₂ of surrounding molecules. These agents are used clinically to decrease the intensity of normal tissue to improve visualization of pathological sections (Figure 1.7).
Figure 1.6: The effect of T1 contrast agents can be seen by comparing the images before (left) and after (right) contrast administration. The boundary of the tumor on the left side of images is highlighted against tissue background with Gd-based contrast. Reference: McRobbie, et al. MRI from Picture to Proton, 2nd ed., p. 43.

Figure 1.7: The SPIO nanoparticles used in this patient results in a decrease in intensity of normal tissue, allowing a tumor to be visualized. Reference: McRobbie, et al. MRI from Picture to Proton, 2nd ed., p. 44.
1.6 Summary of Chapters

Chapter 2 Oxygen sensing
Information on tissue oxygen tension is critical to the successful treatment of many human diseases. Clinical decisions for diseases such as cancer benefit from knowing how oxygen tension changes in response to treatment. We tested several low molecular weight siloxanes for reporting changes in oxygen concentration in the environment. These materials exhibited fast kinetics and reversible changes in response to changes in oxygen concentration.

Chapter 3 Mechanism of sensor response
The performance of MRI contrast agents is typically characterized by their relaxivity. These agents reduce the relaxation times of surrounding molecules, and the intrinsic relaxation times of these molecules cannot be tuned externally. The siloxanes used in this thesis represented a class of contrast agents where the signal can be measured directly. We analyzed the performance of these siloxane molecules and developed a model for oxygen response. The importance of intrinsic relaxation times and other physical parameters that influence the performance of these agents are discussed.

Chapter 4 Solid contrast agents
One of the obstacles preventing the use of MR contrast agents in long-term monitoring applications is their fast clearance rate. The lifetime of these contrast agents can be improved many folds by encapsulating them in a solid polymeric matrix. We developed DDMPS/PDMS microparticles that agglomerate into a solid sensor after injection. The signal from the responsive component, DDMPS, can be isolated with the use of long echo times in a spin-echo pulse sequence.
Chapter 5 Oxygen sensing in animal models
Injected solid contrast agents were tested in two rat models. The inspired gas model validated the oxygen response in the rats and correlated the response to pulse oximeter readings. We also used this model to investigate the performance of injected contrast agents over a one-month period, and found no performance degradation. Test results from a hind-limb ischemia model indicates that the injected contrast agents function independently of blood flow and resisted physical displacement even at elevated pressure levels.

Chapter 6 MR readable pH sensors
Hydrogels with pH-responsive functional groups are useful as pH-responsive materials. These gels have pH-dependent chemical exchange rates with water protons that results in relaxation times that depend on pH values. We synthesized HEMA based hydrogels and incorporated them into microresonator-based sensor devices. These devices responded to changes in pH in a saline bath and detected the acidified pH of tumors in a mouse model.
References


Chapter 2

Oxygen sensing

Oxygen regulates many biological processes in living organisms and plays an important role in pathological processes. An adequate delivery of oxygen to the appropriate tissue is necessary to ensure appropriate energy metabolism, for example. Oxygen monitoring is critical in many areas of clinical medicine and has been used to diagnose different conditions. Current approaches to oxygen sensing are described in this chapter. The advantage of our new approach and its basic operation will be described.

2.1 Motivation

Oxygen is an integral component in energy metabolism, and abnormal oxygen tensions are often indicative of dysregulation in critical metabolic processes. Oxygen tension impacts the progression and treatment response of cancer\(^1,2\), ischemia-reperfusion injury\(^3\), and wound healing\(^4\). An accurate assessment of oxygen tension would help physicians determine the correct diagnosis and treatment choices in the abovementioned examples. The impact of oxygen on different diseases will be discussed in this section.

Two different mechanisms give rise to hypoxia in cancer\(^5\): perfusion limitation and diffusion limitation. Ischemic hypoxia is caused by perfusion-limited oxygen delivery to the tumor tissue, often triggered by lack of oxygen in plasma and of short durations. The second mechanism, chronic hypoxia, is caused by an increase in diffusion distances. Oxygen delivery is directly impacted by how far oxygen has to travel, and cells at a distance greater than \(70 \mu m\) from the capillaries receive
significantly less oxygen than is sufficient for normal metabolic functions\textsuperscript{6}. Both types of hypoxia are found in tumors, and the conditions vary both spatially and temporally. The spatially heterogeneous nature of hypoxia within the tumor means that it is important to sample the same location in the tumor to ensure relevance of results obtained at different time points.

Hypoxia in cancer negatively impacts treatment efficacy and is considered a negative prognostic factor\textsuperscript{7}. Hypoxic tumors have increased metastatic potential and invasiveness, since adaptations that favor survival under hypoxic stress also lead to increase tumor invasiveness\textsuperscript{8}. The association between hypoxia and resistance to treatment is well documented in the radiology field\textsuperscript{9,10}. Oxygen tension in the tumor has been used to predict the success of radiation treatment\textsuperscript{11-13} and is under investigation for the purpose of tailoring the radiation dosage\textsuperscript{14} (Figure 2.1) or timing of therapy administration. Studies have found that three times as much radiation is required to eliminate cancer cells in a hypoxic tumor compared to cancer cells in an oxygenated tumor\textsuperscript{9,15}. Fractionated radiotherapy, the treatment modality where small doses of radiation are administered at multiple time points, could benefit from knowing how oxygen tension evolved in the tumor over time. Performing fractionated radiotherapy at the correct timing requires accurate oxygen measurements in the tumor because tumors tend to become more hypoxic after a radiation dose, and these time periods must be avoided for successful treatment.

Hypoxia has been an attractive target for pharmaceutical interventions in solid tumors, but recent studies have attributed the low efficacy of chemotherapeutic drugs designed to target hypoxia to the lack of a suitable companion diagnostic assay\textsuperscript{16-18}. Cancer patients whose tumors that are not hypoxic would not benefit from a drug designed to target hypoxic cells, and a diagnostic assay for selecting appropriate patients would maximize the probability of drug efficacy. An ideal
companion diagnostic assay would report the oxygen status in the tumor at multiple time points during the course of treatment, as the tumor oxygen tension is dynamic and changes in response to treatment\textsuperscript{19}. The assay would also need to provide quantitative information on oxygen in the tumor to enable correct dosage and patient selection\textsuperscript{2}. Hypoxia is expected in many solid tumors, and quantitative oxygen tension allows for a more systematic approach in segmenting the patients. It is, finally, important for the assay to report the oxygen tension at the same location in the tumor over time. The oxygen distribution in a tumor is heterogeneous, and an assay that samples different locations in the tumor would sample this heterogeneity rather than evolution of oxygen tension over time.

![Graph](image)

**Figure 2.1:** A higher dose of radiation is required to overcome tumor hypoxia in patients with prostate cancer. Specifically, 165 Gy of radiation is required to achieve the same therapeutic outcome compared to 140 Gy in non-hypoxic tumors. Reference: J. Wang, et al., Br J Radiology (2006) 79, p905

Peripheral vascular disease is another condition where knowledge of oxygen tension can improve treatment outcomes. A more precise characterization of the disease based on quantitative oxygen measurements can help physicians optimize treatment regimens. Diabetic foot ulcers, for example, are treated with hyperbaric oxygen therapy with the assumption that sporadic episodes of hypoxia can promote angiogenesis and tissue recovery\textsuperscript{20}. Current treatment regimens are
monitored using transcutaneous oximetry\textsuperscript{21}, a technique that can produce confounding results from regional variations of blood flow. A more quantitative measurement of oxygen tension should allow the physicians to better measure the effectiveness of treatment and make adjustments accordingly.

The concentration of oxygen in wounds plays an important role in the wound healing process\textsuperscript{22}. A continuous supply of oxygen provides resistance to infection and promote the healing process\textsuperscript{23}, and local oxygen therapy has been used to promote healing in situations where blood flow and oxygen supply were compromised. The lack of feedback on how oxygen level has improved, however, makes it difficult to perform treatment adjustments. A tool for accurate assessment of local oxygen level could help establish oxygen therapy as a treatment option for patients recovering from surgery or other injuries\textsuperscript{24}.

2.2 Current Technologies

Technologies currently used for sensing oxygen \textit{in vivo} can be divided into those that directly detect changes in absolute oxygen levels and those that detect relative oxygen tension through changes in concentration of hypoxia markers. The focus in this thesis is on methods that directly detect oxygen concentration. The technologies currently under investigation can be divided into electrochemical, optical, or magnetic resonance methods.

Much of the evidence for hypoxia in solid tumors was obtained using the polarographic oxygen electrode. The Eppendorf Histograph, a computerized polarographic electrode system outfitted with a stepping motor, has been used in the clinic to study human tumors and provided investigators with oxygen tension distributions in the tumors\textsuperscript{18, 25}. The Eppendorf Histograph has become the clinical gold standard for real-time, quantitative measurement of local oxygen
concentration since its introduction, with more than 125 clinical research studies performed using this tool. The polarographic electrode measures oxygen by applying a small potential between the cathode and anode. The reduction of oxygen causes a current, proportional to the concentration of dissolved oxygen, to flow between the electrodes. Measurements with the polarographic electrodes are quick (5-10s to steady state) and quantitative, but these electrodes are not appropriate for chronic monitoring of oxygen tension. The measurement procedure is invasive - even with fine needle electrodes. Repeated measurements in the same area is also challenging due to the need to accurately position the electrode multiple times. The oxygen electrodes require periodic recalibration to correct for long-term instability, a fact that makes the implantation of electrodes impractical. Implanting electrodes also introduces the risk of infection and is a suboptimal choice.

Several related technologies employ optical techniques for measuring oxygen tension. Fluorescence quenching, phosphorescence imaging, and near-infrared spectroscopy (NIRS) are currently under active investigation for clinical use. Fluorescence quenching detects the oxygen concentration directly by examining the oxygen-dependent emission behavior of a fluorophore. This fluorophore is placed at the tip of a fiber optic probe and does not consume oxygen during measurement. The downside to this technique is the accurate placement required of the probe, and repeated measurements at the same location are difficult to achieve. The sensitivity of fiber optic probes is, additionally, limited to oxygen tensions of less than 100 mmHg and is not appropriate for all applications. Phosphorescence imaging is similar to fluorescence quenching in that an oxygen-dependent quenching mechanism is used to quantify oxygen levels. The intensity of light absorbed and reemitted by the phosphorescence material is inversely proportional to the amount of oxygen in tissue and can be calculated from a calibration table. NIRS is similar to pulse oximetry but uses multiple sensors to
enable a greater tissue penetration depth. The technology works by comparing the intensity of light absorbed at specific wavelengths corresponding to hemoglobin and deoxyhemoglobin\textsuperscript{30}. NIRS is susceptible to differences in blood flow and does not provide an independent measurement of tissue oxygen tension.

### 2.3 Magnetic Resonance in Oxygen Sensing

Magnetic resonance represented a non-invasive technology to monitor oxygen tension in the body, and several magnetic resonance imaging (MRI) methods have been developed to detect changes in oxygen tension. Blood oxygen level dependent (BOLD) imaging is a technique currently used in the clinic to examine oxygen consumption patterns in the brain\textsuperscript{31}. The oxygen-dependent contrast is generated through the reduction of transverse relaxation time ($T_2$) by paramagnetic deoxyhemoglobin\textsuperscript{32}. The relative oxygen concentration is then extracted by comparing the measured $T_2$ values in different regions of the image. The drawback of BOLD imaging is the qualitative nature of the technique; BOLD data is influenced by other physiological factors such as pH, temperature, and hematocrit\textsuperscript{31}. These external factors can affect the accuracy of measurement and prevent quantitative information from being extracted.

Electron paramagnetic resonance (EPR) is a technology widely investigated for measuring oxygen tension in the body. This technology uses probes that are implanted or injected into a target tissue site. These probes, in contact with the surrounding tissue, acquire an oxygen concentration that is proportional to that in the tissue. The EPR linewidth of the probes is tied to the amount of oxygen contained in the probe, and the absolute oxygen concentration can be extracted from this linewidth with a calibration curve\textsuperscript{33}. The probes used in EPR are paramagnetic materials in the form of particulates or soluble material\textsuperscript{20}. These probes can be delivered with a small gauge (23 – 26 G) needle and represented a
minimally invasive technology for oxygen sensing. Many EPR probes currently under investigation are inert, have a wide sensitivity range for oxygen (<0.1% - 100% reported), and have demonstrated excellent stability in the body with some probes functioning for up to one year after implantation\textsuperscript{34}. EPR has been tested extensively in preclinical models\textsuperscript{33}, but several challenges needed to be overcome for EPR to gain more widespread use in the clinic. A significant challenge is the depth of tissue assessable to EPR oximetry. EPR typically operates at the gigahertz range (typically 1.2 GHz), which corresponds to tissues that is 8 – 10 mm beneath the surface. Measuring deeper tissue sites with EPR necessitates the use of lower frequencies which decreases the signal to noise ratio. A microresonator could alternatively be surgically implanted\textsuperscript{20}, but this is an invasive procedure that would limit its application. Another limitation of EPR oximetry is the availability of EPR scanners. EPR is only available at research centers and essentially unavailable for use in patients outside of clinical trials\textsuperscript{18}.

2.4 Siloxanes as oxygen contrast agent

Direct observation of oxygen using NMR is difficult because \(^{17}\text{O}\), the only stable and NMR-active isotope, exists at a low (0.038\%) abundance. Direct imaging of oxygen is thus impractical for most clinical applications. The relaxation effect generated by paramagnetic oxygen molecules, however, can be exploited to quantify oxygen concentration. Molecular oxygen has two unpaired electrons in its pi orbital (Figure 2.2) and is paramagnetic. Paramagnetic ions or molecules in solution act as efficient relaxers to reduce the longitudinal relaxation time (\(T_1\)) of neighboring molecules. This increase in relaxation rate is linear with oxygen concentration and is found in any material with dissolved oxygen. Tissue-oxygen-level-dependent (TOLD) contrast is a MRI technique that exploits the change in tissue \(T_1\) caused by dissolved oxygen in tissue and has been used to explore tissue response to changing oxygen concentration in a number of studies\textsuperscript{35-37}. The
oxygen concentrations obtained from these measurements are qualitative in nature as many factors, such as fluctuations in tissue temperature and presence of paramagnetic deoxyhemoglobin, can alter tissue $T_1$\textsuperscript{38}. This method is thus best used for measuring short-term and acute changes in oxygen rather than long-term monitoring.

Figure 2.2: The pi orbital of oxygen molecules contain two unpaired electrons, making the molecule paramagnetic. Reference: Burrows, A. et al., Chemistry\textsuperscript{3}, 2nd ed.

Some of the confounding factors mentioned above, such as changes in tissue perfusion, can be avoided through the use of exogenous contrast agents. The relaxation times of these contrast agents are determined by the material choice and can be tailored for the application. Perfluorocarbons have been used in several preclinical studies for oxygen tension measurements\textsuperscript{39,40}. This technique, also known as $^{19}$F oximetry, is reliable, quantitative, and correlates well with established methods such as BOLD or oxygen electrodes\textsuperscript{41,42}. One of the distinguishing factors of $^{19}$F oximetry is the lack of $^{19}$F background signals in the body. The obtained NMR signals would therefore consist solely of signal from the contrast agents. Clinical translation of $^{19}$F technology remains difficult, however,
because of the lack of appropriate instrumentation for performing $^{19}\text{F}$ imaging sessions.

Most clinical MRI machines are tuned to the proton resonant frequency. Contrast agents designed to work at the proton resonant frequency would thus have the biggest clinical translation potential. Kodibagkar et al. recently used Hexamethyldisiloxane (HMDSO) to detect changes in tissue oxygen level in a mouse model with $^1\text{H}$ MRI. The measurement results produced with HMDSO were quantitative and correlated well with $^{19}\text{F}$ oximetry results thus making this class of chemicals a useful alternative to $^{19}\text{F}$ oximetry. Siloxanes have enjoyed a long history of use as a medical material. Liquid siloxanes have been used as a filler in cosmetic procedures and a 1000cst silicone oil was recently approved for use in the treatment of retina detachment. The toxicity of siloxanes is well studied, and the material is generally considered safe and non-toxic.

We investigated the oxygen response of four different linear siloxanes (Figure 2.3). Oxygen response, as used here, is defined as the difference in longitudinal relaxation times ($T_1$) at different oxygen partial pressures. Relaxation times were acquired using the Bruker Minispec mq20 system (0.5T and 40°C, Bruker Biosciences, MA, USA). The samples were pre-heated to 40°C prior to measurements to ensure a consistent sample temperature. Acquisition of $T_1$ was performed with an inversion recovery sequence with the following parameters: echo time (TE) = 0.5 ms, 4 scans, 13 inversion points, and TR = 5 times measured $T_1$. The data generated was mapped to the inversion recovery equation $I = I_o(1 - 2e^{-t/T_1})$ using a custom script running on MATLAB (The Mathworks, MA, USA). The oxygen partial pressure in the sample environment was modulated using a gas mixer (Pegasus Systems) system.
The siloxane molecules all showed reversible response to step changes in oxygen partial pressure. The $T_1$ data shows that absolute $T_1$ values scale inversely with siloxane molecule size. This difference is more pronounced at low oxygen partial pressures and reflects the large differences in intrinsic relaxation time between siloxanes. The samples responded to changes in oxygen partial pressure within 10 minutes of changes in oxygen partial pressure (Figure 2.4) and demonstrated excellent reversibility over multiple cycles of gas switches. The sensitivity of these materials is the highest at low oxygen partial pressures (Figure 2.5). This property makes them suitable for use in the body and especially in situations where hypoxia is expected.
Figure 2.4: The measured longitudinal relaxation times of siloxanes (top) equilibrate to changes in oxygen partial pressure (bottom) within 10 minutes. The response is completely reversible after multiple cycles of gas switches.
The change in the $T_1$ of siloxanes is greatest at low oxygen partial pressures and corresponds to the oxygen concentrations typically encountered in the body.

The change in relaxation time is caused by interaction with paramagnetic oxygen molecules and the relaxation rates are linearly proportional with oxygen concentration. This can be seen when $\frac{1}{T_{1,\text{measured}}}$, the reciprocal of $T_1$, is plotted against the oxygen partial pressure in Figure 2.6. The y-intercept on the plot represents the intrinsic relaxation rate and the slope represents the change in relaxation rate with oxygen partial pressure.
Figure 2.6: The longitudinal relaxation rate is linear with oxygen partial pressure. Each data point represents an average of 6 measurements and the measurements were performed at 40°C.

2.5 Conclusions

The use of low molecular weight siloxanes for quantitative oxygen sensing was demonstrated. The dissolved oxygen molecules act as diffusing paramagnetic species in these siloxanes, and the longitudinal relaxation times of the siloxanes reflect the oxygen concentration. The oxygen response is fast, reversible, and correlates to the oxygen partial pressure in the environment. The relaxation time of the molecules scale inversely with molecule size, a fact that allows the sensitivity and study time to be tuned.
References


The performance of magnetic resonance imaging (MRI) contrast agents is typically characterized by their relaxivity. These agents reduce the relaxation times of surrounding molecules, and the intrinsic relaxation times of these molecules do not affect the measurement results. We discuss in this paper the characterization of a class of contrast agents where both the intrinsic relaxation times and an analyte-dependent relaxivity are under user control. The signal from these agents is directly recorded by MRI. The importance of intrinsic relaxation times and other physical parameters that influence the performance of these agents are discussed. We focused on the response to oxygen in this paper, but the principles described could be useful for the design of similar responsive contrast agents such as those used in $^{19}$F MRI.

### 3.1 Theory
The signal intensity at a given recovery time, $\tau$, is given by:

$$I = 1 - 2e^{-\frac{t}{T_1}}$$

Relaxation times are extracted using signal intensity measurements at multiple recovery times. The sensitivity of these measurements is dependent on the maximal change in relaxation time and the precision with which that relaxation time can be measured. The total available signal is given by the difference between the two extreme relaxation times:
\[ S = \int I_1 - I_2 \, dt = \int_0^\infty 2 \left( e^{\frac{-t}{T_1 + \Delta T}} - e^{\frac{-t}{T_1}} \right) \, dt \]
\[
S = 2\Delta T \propto \Delta T
\]

The available signal is proportional to the difference in relaxation times. Practically only a finite number of recovery times are used and this integral represents an upper bound on the available signal.

The noise in the measurement must also be considered, because the sensitivity is determined by how accurately the relaxation times can be measured. The noise is typically reduced by averaging multiple repetitions of the same measurement. The number of measurements that can be performed in a fixed amount of time is inversely proportional to the intrinsic \( T_1 \) of the material, because the longitudinal magnetization must be allowed to recover after each measurement. More scans can be taken with materials with short \( T_1 \) in a given period of time, because the recovery delay time can be shorter with these materials. Averaging these scans reduces the overall noise in the measurement. The signal-to-noise ratio is:

\[ SNR \propto \frac{\Delta T}{\sqrt{T_1}} \]

Relaxation time measurements are acquired with a spin-echo sequence in our experiments. These measurements involve rotating the magnetization into the transverse plane. The signal decays at a rate inversely proportional to the transverse relaxation time (\( T_2 \)) of the material. The signal-to-noise ratio is scaled by a factor of \( T_2^{1/2} \) to give:
\[ SNR \propto \frac{\Delta T}{\sqrt{T_1}} \cdot \sqrt{T_2} \]

The SNR is also proportional to the fitting process used to estimate \( T_1 \). The relaxation times are obtained by fitting the acquired intensities to the inversion recovery equation with least-squares fitting, and the amount of noise propagated into the \( T_1 \) estimates is proportional to the number of points used to fit the data\(^2\). This deviation in \( T_1 \) fitting, \( \sigma_{T_1} \), can be incorporated into the SNR expression:

\[ SNR \propto \frac{\Delta T}{\sigma_{T_1}} \cdot \frac{\sqrt{T_2}}{\sqrt{T_1}} \]

The factor \( \sigma_{T_1} \) is set to unity for ease of comparison since the number of data points used can be made to be the same for different samples. We note that the terms in the SNR equation can be classified into two factors: a factor determined by the oxygen response, \( \Delta T \), and a factor determined by the intrinsic material properties, \( \frac{T_2}{\sqrt{T_1}} \). The latter can be measured directly on a neat sample of the material.

We are interested in comparing the relaxation time of a given material between the oxygenated and deoxygenated conditions. \( \Delta T \) is related to both the intrinsic material relaxation rate and an oxygen-dependent relaxation rate. The relaxation rate of a material with multiple components can be expressed as a sum of relaxation rates of those components. The longitudinal relaxation rate of a material with dissolved oxygen can be broken down into a sum of the intrinsic material relaxation rate, \( R_{1,\text{mat}} \), and an oxygen-dependent relaxation term, \( R_{1,\text{ox}} ([O2]) \):
\[
\frac{1}{T_1} = R_1 = R_{1,\text{mat}} + R_{1,\text{ox}}([O2])
\]

The \(R_{1,\text{ox}}\) term can be modeled similarly to diffusing paramagnetic ions in solution; this effect can be approximated with the following equation\(^3\):

\[
R_{1,\text{ox}} = \frac{16\pi^2}{15} \cdot \langle \mu^2 \rangle \cdot \frac{\gamma^2}{kT} \cdot N_{\text{oxygen}} \cdot \eta
\]

where \(\langle \mu \rangle\) is the effective magnetic moment of oxygen, \(\gamma\) is the hydrogen gyromagnetic ratio, and \(k\) is the Boltzmann constant. Oxygen solubility in the material is represented by \(N_{\text{oxygen}}\), the concentration of dissolved oxygen. The sample viscosity is represented by \(\eta\). The equation can be rewritten as a function of oxygen partial pressure using Henry’s law:

\[
R_{1,\text{ox}} = \frac{16\pi^2}{15} \cdot \langle \mu^2 \rangle \cdot \frac{\gamma^2}{kT} \cdot \frac{\eta}{H} \cdot P_{O_2}
\]

where \(H\) is Henry’s law constant, the reciprocal of gas solubility.

This equation can be rewritten as a function of oxygen diffusivity using the Stokes-Einstein relation. The assumptions here are that the ions have point dipoles in the center, and that the effective magnetic moment \(\mu\) is calculated assuming that the particles cannot overlap. The equation then becomes:

\[
R_{1,\text{ox}} = \frac{4\pi}{5} \cdot \gamma^2 \cdot \langle \mu^2 \rangle \cdot N_{\text{oxygen}} \cdot \frac{1}{(r_{ox} + r_s)(D_{ox} + D_s)}
\]
where \( r_{ox} + r_s \) represents the closest distance of approach between a siloxane molecule and oxygen molecule, and \( D_{ox} + D_s \) are the diffusivity of oxygen and the siloxane molecule.

\( \Delta T \) can now be expanded using the relaxation rate equations described above. \( \Delta T \) is the difference in \( T_1 \) measured at two different oxygen tensions, and any two oxygen tensions can be chosen for a comparison. The difference is, however, maximized when one of the measurements is obtained when the material is deoxygenated:

\[
\Delta T = T_{1,\text{mat}} - T_{1,02}
\]

\[
= \frac{R_{1,ox}}{R_{1,\text{mat}}^2 + R_{1,\text{mat}} \cdot R_{1,ox}}
\]

This expression for \( \Delta T \) can be substituted back into the SNR equation and simplified:

\[
\text{SNR} \propto \frac{T_{1,\text{mat}} \cdot R_{1,ox}}{1 + T_{1,\text{mat}} \cdot R_{1,ox}} \cdot \sqrt{T_{1,\text{mat}} T_{2,\text{mat}}}
\]

\[
= \frac{1}{1 + \frac{1}{\tau_{1,\text{mat}} \cdot R_{1,ox}}} \cdot \sqrt{T_{1,\text{mat}} T_{2,\text{mat}}}
\]

The maximum value of the SNR is determined by \( \sqrt{T_{1,\text{mat}} T_{2,\text{mat}}} \) and is achieved if the product \( T_{1,\text{mat}} \cdot R_{1,ox} \) is much greater than unity. This represents a material that has a very long intrinsic \( T_1 \) and a large contribution of oxygen on that relaxation time.
3.2 Experiments

We measured relaxation times for this study because these variables directly interrogate changes introduced by oxygen. Image contrast is susceptible to changes in imaging and instrument parameters, and relaxation times represented a more robust way to acquire quantitative information.

Proton relaxation times were measured using a bench top NMR relaxometer (Bruker Minispec mq-20) with a 0.47 T static field. Longitudinal relaxation time ($T_1$) measurements were conducted using an inversion recovery pulse sequence with the signal intensity measured at 13 recovery times. Intensity data, averaged over four scans, were fitted to the equation $I = I_0 (1 - 2e^{-t/T_1})$ to extract the relaxation time. The recovery delay time was varied between samples and was set to five times the measured relaxation time to ensure complete spin relaxation. Transverse relaxation times ($T_2$) were acquired using a standard multi-echo pulse sequence (CPMG) with the following parameters: echo time = 500 µs, TR = 2000 ms, number of scans = 4. The resulting echo amplitudes were fitted to the equation $I = I_0 e^{-t/T_2}$ to extract the transverse relaxation time.

Magnetic Resonance Imaging was performed using a 7 T small animal imager (Agilent, USA). The imaging protocol includes a scout pulse sequence for locating devices and a fast spin-echo sequence with inversion recovery for performing $T_1$ measurements with the following parameters: matrix size = 64 x 64, FoV = 32 mm x 32 mm, slice thickness = 2 mm, TE = 12.5 ms, ETL = 16, and kzero = 8. The effective TE of sequence used was 100 ms. TR was adjusted depending on the $T_1$ of particular samples and was set to at least 3x measured $T_1$. Eight inversion times, separated exponentially, were used to generate data for $T_1$ mapping. The measurements were performed in a quadrature volume coil that measures 63mm in diameter.
Oxygen solubility was measured using gas chromatography (GC) with a protocol first described by Wesseler et al⁴. The samples were bubbled with oxygen for 20 minutes prior to injection into the GC machine. A recirculating water bath maintained the samples at a constant temperature of 40°C.

Oxygen diffusivity was measured using a NMR method developed in our lab. Samples were loaded in a glass vial and equilibrated in nitrogen. A single-sided NMR relaxometer (NMR MOUSE) measured intensity of a thin slice at a set distance into the sample. Changes in intensity, introduced by the presence of oxygen, were used as a surrogate for relaxation time to enable fast measurements. These intensity values were then mapped to diffusion equations to calculate the diffusivity.

### 3.3 Components of sensor response

Work on the characterization and optimization of metal-ion based contrast agents have focused on tuning the relaxivity of the contrast agents⁵ because those agents are designed to increase the relaxivity of surrounding molecules while contributing negligibly to the overall signal. The contrast agents discussed here, on the other hand, are similar to $^{19}$F MR contrast agents and contain protons that can be imaged by conventional magnetic resonance imagers. The measured relaxation rate of any material with dissolved oxygen is a sum of the intrinsic relaxation rate, $R_{1,mat}$, and an oxygen dependent relaxation rate, $R_{1,ox}$. SNR of the material, proportional to the maximal change in relaxation time with change in oxygen concentration, is derived in section 3.1 and given as the following equation:

$$SNR \propto \frac{1}{1 + \frac{1}{T_{1,mat} R_{1,ox}} \cdot \sqrt{T_{1,mat} T_{2,mat}}}$$ (1)
where $T_{1,\text{mat}}$ is the intrinsic relaxation time and $T_{2,\text{mat}}$ is the intrinsic transverse relaxation time of the material.

### 3.3.1 Intrinsic Relaxation time

The relationship between intrinsic relaxation time and expected SNR can be seen in Figure 3.1. $T_{1,\text{mat}}$ can be used to predict the SNR of the materials and establishes a ceiling value for SNR (Table 3.1). If the product $T_{1,\text{mat}} \cdot R_{1,\text{ox}}$ is much greater than 1, the SNR becomes roughly equal to $\sqrt{T_{1,\text{mat}} T_{2,\text{mat}}}$. The values of $T_{1,\text{mat}}$ and $T_{2,\text{mat}}$ are the same in these materials, giving us $\text{SNR} \propto T_{1,\text{mat}}$ and a linear relationship between SNR and $T_{1,\text{mat}}$. The fast molecular motion and low correlation time found in these materials lead to $T_{1,\text{mat}}$ and $T_{2,\text{mat}}$ being the same.$^6$

Oxygen contribution can be used as a metric for materials with similar intrinsic relaxation times, but is not directly predictive of overall SNR. $T_1$ relaxation is driven by random magnetic field fluctuations and molecular interactions in the lattice.$^7$ This interaction, characterized by the correlation time between molecules, results in small molecules having longer relaxation times.$^8$
Figure 3.1: SNR vs T1, mat. This plots shows the relationship between calculated SNR and the intrinsic T1. DDMPS, DMOO, and DI water have different oxygen contributions. This difference is highlighted by an increase in SNR from DI water to DDMPS. The dotted line represents the maximal SNR possible at a particular relaxation time that is achieved when the contribution of oxygen brings the relaxation time to 0. Relaxation times were obtained on a benchtop NMR relaxometer.

T1 relaxation is driven by random magnetic field fluctuations and molecular interactions in the lattice. The most important relaxation mechanism in spin-1/2 (proton) systems is dipolar coupling, the interaction between two magnetic dipoles. Dipolar interaction is characterized by the correlation time $\tau_c$, the time it takes for a molecule to rotate by 1 radian. Correlation time increases with molecular weight and solution viscosity, and the longitudinal relaxation time is at a minimum when the inverse of correlation time (molecular interaction rate) is close to the Lamor frequency. The average molecular interaction rate of small
molecules is much higher than the Larmor frequency, and only a small fraction of molecules interact at the frequency that produces efficient longitudinal relaxation. Thus small molecules have long $T_1$ values. Molecular motion become slower and more efficient at inducing relaxation because the correlation time increases with molecular size or solution viscosity. $T_1$ increases with molecular size again when the average molecular motion becomes slower than the Larmor frequency as can be seen in the cases of some solids (Figure 3.2).

![Diagram](https://via.placeholder.com/150)

Figure 3.2: The longitudinal and transverse relaxation time of molecules as a function of correlation time. Figured reproduced from Bloembergen, E.M. Purcell, R.V. Pound "Relaxation Effects in Nuclear Magnetic Resonance Absorption" *Physical Review* 1948, 73, 679-746

The transverse relaxation time is driven by random molecular motion and has a monotonic relationship with correlation time. The anisotropic motion present in small molecules means that motional averaging would cancel out much of the effects from dipolar interaction, resulting in a high relaxation time. There are fewer degrees of freedom of molecular motion allowed in polymers systems; thus, the correlation time in these systems are higher than small molecules$^{10}$. Specific molecular architectures, such as ring structures, have restricted rotational movement compared to linear structures and thus a lower relaxation time.
<table>
<thead>
<tr>
<th>Group</th>
<th>Chemical</th>
<th>MW</th>
<th>( T_{1,\text{oxy}} ) [ms]</th>
<th>( T_{1,\text{deoxy}} ) [ms]</th>
<th>( T_{2,\text{oxy}} ) [ms]</th>
<th>( T_{2,\text{deoxy}} ) [ms]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linear Siloxanes</td>
<td>HMDSO</td>
<td>161.39</td>
<td>555</td>
<td>8958</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>OMTSO</td>
<td>236.53</td>
<td>488</td>
<td>6483</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>DMTSO</td>
<td>310.69</td>
<td>471</td>
<td>5050</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>DDMPS</td>
<td>384.84</td>
<td>456</td>
<td>4150</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Cyclic siloxanes</td>
<td>OMCTS</td>
<td>296.62</td>
<td>365</td>
<td>2972</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Non-polar</td>
<td>DMCPS</td>
<td>370.77</td>
<td>365</td>
<td>2384</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>Hexane</td>
<td>86.18</td>
<td>745</td>
<td>9095</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Polar</td>
<td>DMSO</td>
<td>78.13</td>
<td>1160</td>
<td>4131</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>DI Water</td>
<td>18.02</td>
<td>2009</td>
<td>4566</td>
<td>*</td>
<td>*</td>
</tr>
</tbody>
</table>

Table 3.1: Relaxation times of materials measured at 1 atm oxygen and deoxygenated state. * denotes transverse relaxation times that are substantially similar to longitudinal relaxation times. \( T_{1,\text{mat}} \) correlates inversely with molecular weight both within a family of materials and between different material systems. Siloxanes with ring structures have a lower intrinsic relaxation time compared to linear siloxanes; the intrinsic relaxation time of OMCTS, for example, is about 40% lower than that of DMTSO even though their molecular weights are similar.

3.3.2 Oxygen contribution to relaxation time

The oxygen contribution to relaxation time, \( R_{1,\text{ox}} = R_{1,\text{measured}} - R_{1,\text{mat}} \), is calculated from relaxation time measurements and is not directly measurable. This variable can, however, be expressed as a function of oxygen solubility and oxygen diffusivity from section 3.1:

\[
R_{1,\text{ox}} = \frac{4\pi}{5} \cdot \gamma^2 \cdot \langle \mu^2 \rangle \cdot N_{\text{oxygen}} \cdot \frac{1}{(r_{\text{ox}} + r_s)(D_{\text{ox}} + D_s)} \quad (2)
\]

where \( \langle \mu \rangle \) is the effective magnetic moment of oxygen, \( \gamma \) is the hydrogen gyromagnetic ratio, and \( k \) is the Boltzmann constant. Oxygen solubility in the material is represented by \( N_{\text{oxygen}} \), the concentration of dissolved oxygen. \( r_{\text{ox}} + r_s \) determines the closest distance of approach between a siloxane molecule and oxygen molecule, and \( D_{\text{ox}} + D_s \) are the diffusivity of oxygen and the self-diffusivity of the siloxane molecule. The self-diffusion coefficients of linear siloxanes were measured experimentally by McCall et al.\(^{11} \). The oxygen diffusivity coefficients in liquid siloxanes were calculated with the Stokes-Einstein
relation from published viscosity values, whereas the oxygen diffusivity in the matrix embedded siloxane composites were measured by measuring relaxation changes with NMR as the oxygen diffuses into the material.

Equation 2 suggests that $R_{1,ox}$ would be maximized with large oxygen solubility and low oxygen diffusivity. The oxygen contribution of both the linear siloxanes and matrix embedded siloxanes scale linearly with the ratio $\frac{N_{oxygen}}{D_{ox}+D_s}$ (Figure 3.3), as predicted by equation 2. We expected minimal changes in self-diffusion coefficients and oxygen diffusivity by embedding the siloxanes in PDMS. The oxygen contributions are similar between embedded and neat LMW siloxanes. The discrepancy is likely due to the different methods required to measure viscosity in a neat liquid and diffusivity in the PDMS embedded material.

The properties of these solids are driven by the solvent contained within the PDMS matrix under the long echo time condition, and we have characterized additional solvents to gain a more complete picture of different physical properties affecting the oxygen contribution.
Figure 3.3: Correlation between oxygen solubility and diffusivity of solid LMW siloxanes in PDMS at 70% w/w and liquid siloxanes. The oxygen solubility of matrix embedded siloxanes were scaled to compensate for effectively smaller volume. Both neat and matrix-embedded siloxanes respond linearly as predicted by the oxygen response equation. Relaxation times were measured with MRI with an echo time of 100 ms.

Figure 3.4 shows a plot of oxygen solubility versus $R_{1,ox}$. Oxygen solubility reflects the amount of dissolved paramagnetic oxygen in the material. The most important factor affecting oxygen solubility is the polarity of the material tested. We found that the solubility of oxygen increases with decreasing solvent polarity. Molecular oxygen is nonpolar and its solubility is expected to vary inversely with solvent polarity $^{12,13}$.

The regular solution model offers some insight into the trend of oxygen solubility in the group of siloxanes (Table 3.2). The key points gleaned from this theory are that the amount of dissolved oxygen allowed in the material is inversely proportional to the intermolecular forces in the material, and that decreased intermolecular forces are associated with increased volatility $^{14-16}$. These conclusions are consistent with our observations that oxygen solubility is the
highest in the most volatile siloxane molecules, which have the shortest chain lengths.

Figure 3.4: Linear fit of oxygen solubility of solvents and oxygen contribution to the relaxivity. Polarity strongly predicts oxygen solubility as can be seen in the plot. The oxygen solubility of hexane and DI water, for example, varied by an order of magnitude with DMSO in between the two. The cluster of siloxane samples on the right side of the plot illustrates that samples with different viscosity plays a more significant role when comparing materials with similar oxygen solubility. Relaxation times were obtained a benchtop NMR relaxometer.

We plotted $\frac{N_{\text{oxygen}}}{D_{\text{oxygen}} + D_{\text{s}}}$ against $R_{1,\text{ox}}$ in Figure 3.5 for the rest of the solvents. It can be seen that the relationship is linear for solvents within a particular chemical class, but the relationship fails when comparison across different chemical types were made. These underlying assumption that the correlation time between oxygen and the molecules are similar, while true for similar molecules, would not work for molecules of dissimilar nature\(^{17}\). Other relevant parameters, such as the distance of
The closest approach between the paramagnetic ion and nucleus, can be measured to improve these correlations.

Figure 3.5: The oxygen contribution to the relaxivity, $R_{1,ox}$, plotted against the oxygen solubility / total diffusivity. The different material groups can be seen to follow the trend of increasing oxygen contribution with a larger product of oxygen solubility or lower oxygen diffusivity. Relaxation times were measured on a benchtop NMR relaxometer.

<table>
<thead>
<tr>
<th>Group</th>
<th>Chemical</th>
<th>Oxygen solubility [mL/mL]</th>
<th>Viscosity [cst]</th>
<th>$R_{1,ox}$ [s$^{-1}$]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linear Siloxanes</td>
<td>HMDSO</td>
<td>0.387</td>
<td>0.65</td>
<td>1.69</td>
</tr>
<tr>
<td></td>
<td>OMTSO</td>
<td>0.363</td>
<td>1</td>
<td>1.89</td>
</tr>
<tr>
<td></td>
<td>DMTSO</td>
<td>0.359</td>
<td>1.5</td>
<td>1.93</td>
</tr>
<tr>
<td></td>
<td>DDMPS</td>
<td>0.330</td>
<td>2</td>
<td>1.95</td>
</tr>
<tr>
<td>Cyclic Siloxanes</td>
<td>OMCTS</td>
<td>0.333</td>
<td>2.2</td>
<td>2.40</td>
</tr>
<tr>
<td></td>
<td>DMCTS</td>
<td>0.326</td>
<td>3.8</td>
<td>2.32</td>
</tr>
<tr>
<td>Non-polar</td>
<td>Hexane</td>
<td>0.259</td>
<td>0.3</td>
<td>1.23</td>
</tr>
<tr>
<td>Polar</td>
<td>DMSO</td>
<td>0.041</td>
<td>2</td>
<td>0.62</td>
</tr>
<tr>
<td></td>
<td>DI Water</td>
<td>0.005</td>
<td>1</td>
<td>0.28</td>
</tr>
</tbody>
</table>

Table 3.2: Physical parameters of materials tested
3.3.3 Transverse relaxation time
A difference in transverse relaxation time is needed to eliminate the signal from the PDMS matrix; otherwise, a double-exponential fit is required. $T_2$ is inversely related to viscosity, the size of the molecule, and restricted molecular motion in the environment. The transverse relaxation time changes with oxygen in most materials. The SNR expression presented in the theory section overestimates the SNR because the $T_2$ oxygen dependency was not taken into account. The effect of $T_2$ becomes important in solids or heavily cross-linked polymer materials, such as crosslinked PDMS. The $T_1$ in these materials can be substantially longer than $T_2$, and the SNR is really limited by the $T_2$ in these materials.

3.4 Optimization strategy
The relaxivity and concentration of the contrast agent (oxygen) is captured by the $R_{1,ox}$ term in our SNR equation. The intrinsic relaxation time, on the other hand, determines the minimum oxygen contribution required for an acceptable SNR and the maximum signal (see equation 1). Maximizing the intrinsic relaxation time results in an overall more sensitive material.

A strategy to optimize oxygen sensitivity is illustrated using examples with different $T_{1,mat}$ and $R_{1,ox}$. The importance of $T_{1,mat}$ is apparent when comparing the SNR of DI water and the cyclic siloxanes. The intrinsic relaxation time of DI water is about 2 times higher than that of DMCPS, and the oxygen contribution of DI water is about 8 times lower than DMCPS with an oxygen solubility that is about 65 folds lower. The overall SNR of DI water is about 1.3 times higher than that of DMCPS. $T_{1,mat}$ should therefore be considered a strong predictor of material performance. The effect of differences in $R_{1,ox}$ is significant only in materials with similar $T_{1,mat}$, such as DMSO and DDMPS. Another example can
be seen in the performance of hexane – its oxygen solubility and indeed the oxygen contribution is lower than all low molecular weight siloxanes studied in this paper, but the SNR of hexane is comparable with HMDSO, the siloxane with the highest oxygen solubility. This result also strongly supports the use of $T_{1,\text{mat}}$ as the primary benchmark for new materials development.

$T_{1,\text{mat}}$ is determined by the correlation time between molecules in the system. Materials with low molecular weight and low viscosity have low correlation times and thus high intrinsic relaxation times. Some applications require the use of polymeric materials. A robust structure, specifically, is needed for materials that are exposed to harsh environments or organic solvents. Crosslinked polymers represent a class of material that can withstand these environments without performance degradation. Polymer samples, however, tend to have low transverse relaxation times. Strategies that increase motion of polymer chains, such as minimizing the molecular weight of subunits and the number of crosslinks, can be used to optimize these materials.

Introducing specific interaction with oxygen can increase $R_{1,\text{ox}}$. A specific solvent-oxygen interaction can, for example, selectively increase the correlation time between solvent and oxygen. A high intrinsic relaxation time and high oxygen response can then both be achieved. This will likely involve a material that changes conformation (i.e. Hemoglobin) or local viscosity with the presence of oxygen. This local interaction needs to be balanced out with the correct association/dissociation constants so as to achieve a fast and reversible sensing functionality.
3.5 Conclusion

We have identified key physical characteristics that are important to understanding the signal-to-noise ratio in oxygen-responsive contrast agents. The relationships uncovered here can be generalized to materials that depend on extrinsic additions of paramagnetic species or in materials where the relaxation time is proportional to the concentration of analytes. The readout mechanism for this class of materials is different from clinically used contrast agents. This signal originated from the contrast agents themselves, instead of reducing the relaxation times of surrounding water protons. The intrinsic relaxation time of these materials, in particular, is a key parameter that determines how sensitive measurements can be.
References


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Chapter 4

Solid State Contrast Agents

This chapter describes a novel technology for retaining contrast agents in vivo. Conventional contrast agents for MRI are liquid materials with little molecular specificity, which are useful for highlighting specific pathological features but do not provide chemical information of the environment. Responsive contrast agents, many of which are under active investigation, provide analyte-dependent contrast, but the time-varying concentration of these agents creates uncertainty in measurements. A solid contrast agent that is retained in the tissue site of interest for an extended period of time can be used to provide long-term monitoring of metabolites.

4.1 Motivation

Magnetic Resonance Imaging (MRI) is one of the most important clinical technologies for monitoring disease progression. MRI is unique amongst imaging modalities in its ability to gather both high-resolution anatomical images and physiological information in the same study\(^1\). Targeted contrast agents are useful for highlighting specific molecules in the body\(^2\), whereas responsive contrast agents enable quantitative diagnostics using MRI. The concentration of contrast agents decreases over time due to systemic clearance\(^3\); some MR methods, such as dynamic contrast-enhanced imaging, exploit this clearance of contrast agent to make inferences about the underlying physiology. Some applications, however, require a consistent concentration of contrast agent for repeatable, accurate and quantitative measurements\(^4\), and an internal control must be added to account for the time-changing concentration. Repeat measurements to track the progression of
disease or the response to treatment also require a new bolus injection of contrast agent. These additional injections introduce uncertainty in the measurements, and the safety of long-term exposure to the contrast agent must be investigated.

Indeed, a recent review described the changing concentration of responsive contrast agents to be a major obstacle to their increased adoption in the clinic\(^4\). The current strategies designed to overcome the varying contrast agent concentration have focused on the use of an external standard to establish the contrast agent concentration. ML Garcia-Martin et al.\(^5\) used simultaneous injections of two molecules, one sensitive to pH changes and another one insensitive to pH changes, with similar pharmacokinetic profiles to create a system for measuring pH values using MRI. The concentration of the pH-sensitive agent is inferred from the measured concentration of the pH-insensitive agent. The accuracy of this method depends heavily on the similar clearance profiles of the two injected molecules. Disturbances in blood flow and drug metabolism can lead to inaccurate results and present incorrect conclusions.

We describe a novel method to ensure consistent measurement of contrast agents. The oxygen-sensitive contrast agents, described in chapter 2, are encapsulated with a polymeric matrix to ensure the retention of these agents in the target tissue for extended periods of time. Quantitative measurements can be performed without concern for baseline shifts or performance degradation because the concentration of contrast material is kept constant. This thesis focuses on the development of oxygen-sensitive contrast agents, but incorporation of other “smart” contrast agents, such as those designed to respond to changes in pH\(^6\) and specific enzymatic reactions\(^7\), is an interesting direction for this research in the future.
4.2 Siloxane/PDMS Oxygen sensors

The contrast material is divided into two components. The first component is a polymeric matrix that provides the physical structure, and the second component is a responsive material that generates the response to a target analyte. This two component system allows the structural element and sensing element to be optimized independently: the matrix can be selected for its physical properties and its ability to retain the sensing agent, and the responsive material can be chosen to optimize the response kinetics and sensitivity. A variety of pulse sequence allows signal from the responsive material to be isolated from the matrix material. No additional controls are needed and repeat measurements on the same location are possible since the depot secures the contrast agent and the contrast agent concentration remains constant. Low molecular weight siloxanes (described in chapter 2) will be used as the responsive material in this chapter unless noted otherwise.

The matrix for supporting the contrast agent must not cause an adverse tissue response. Polydimethylsiloxane (PDMS) was chosen as a suitable polymeric matrix for the siloxanes. It is biocompatible, inert, and amenable to a variety of fabrication methods. PDMS is also highly permeable to oxygen, miscible with the linear siloxanes, and is not expected to inhibit oxygen diffusion into the siloxane. Silicones are some of the most widely used polymeric compounds in the medical community. Polydimethylsiloxane (PDMS, Figure 4.1), in particular, is known for its structural flexibility with a low glass transition temperature (approximately -130°C), and has been widely used in the biotechnology and medical industries due to its excellent biocompatibility and inertness.
PDMS has been used as orthopedic implants, catheters, drains, shunts, and plastic surgery implants. PDMS has been verified by other researchers to stay functional and stable in vivo for extended periods of time\(^9,10\), and has been approved by the FDA for implantation. PDMS also has one of the highest oxygen permeability\(^11\) amongst different polymer systems. This high oxygen permeability, coupled with biocompatibility, makes PDMS an excellent choice for use as a matrix material in our oxygen sensing system.

PDMS can be synthesized with different functional groups. The variant of PDMS we used for enclosing the contrast agents was the commercially available Sylgard 184 Elastomer kit (Dow Corning, MI, USA). This material cross-links into a solid elastomer when heat-cured with a platinum catalyst. The curing process is unaffected by the presence of unreactive fillers that were pre-mixed prior to polymerization. Other siloxanes can, therefore, be added to the pre-polymer mixture without affecting the curing process. Solid sensors were fabricated by physically mixing linear siloxanes with Sylgard 184 prior to heat curing at 80°C for 90 minutes. Devices of different sizes and shapes can be made with the use of different molds (Figure 4.2). A change in device design with this fabrication scheme requires only minimal changes to existing process parameters and tooling compared to prefabricated devices. The finished devices can be loaded into hypodermic needles or biopsy needles for injection.
Properties of the ideal oxygen-sensitive contrast agent would be driven by the application. The tradeoff between sensitivity and study time is especially important. Typical scan times (TR) for $T_1$ measurements are 3 to 5 times longer than the expected $T_1$ (to ensure maximal signal recovery), whereas the sensitivity to oxygen tension is proportional to $T_1$. It is thus practical to use a material with the shortest $T_1$ while satisfying the sensitivity requirements for the application. Doedecamethylpentasiloxane (DDMPS) was selected as the contrast agent for our studies owing to its short relaxation time compared to other low molecular weight siloxanes. The shorter longitudinal relaxation time of DDMPS means that studies could be completed faster and complications from anesthesia could be minimized. DDMPS is also the least volatile material in the group of siloxanes tested, and the low volatility contributes to easier processing and storage of finished devices.

The responses of PDMS devices with different amounts of DDMPS were characterized by measuring the longitudinal relaxation times at different oxygen partial pressures. Longitudinal relaxation times were measured using a benchtop NMR relaxometer (Bruker Minispec mq20) with a 0.5 T static field. Relaxation times on the Minispec were measured using an inversion recovery pulse sequence with the signal intensity measured at 13 recovery times. Intensity data averaged
over 4 scans were fitted to the equation \( I = I_0 (1 - 2e^{-\frac{t}{\tau_1}}) \) to extract the relaxation time.

The observed relaxation times were dominated by the amount of DDMPS in the devices, as evidenced by their correlation (Figure 4.3). A larger percentage of DDMPS in the mixture resulted in a higher relaxation time and greater difference in relaxation time between different oxygen partial pressures. This result suggests that oxygen responsiveness stems from the presence of DDMPS rather than the PDMS matrix. The amount of DDMPS in the PDMS matrix does not alter the kinetics of response to changing oxygen concentrations, and the sensors equilibrated within 10 minutes of a change of oxygen partial pressure.

![Figure 4.3: Both the longitudinal relaxation time and the change in relaxation time are proportional to the amount of DDMPS embedded in the PDMS matrix. The response was reversible and equilibrates within 10 minutes of gas change.](image)
Repeatable results are important for any sensing system, and the baseline stability of implantable sensors is particularly important since baseline drifts create uncertainties in measurements. A decrease in DDMPS concentration in the PDMS matrix, for example, would result in a reduced relaxation time at any given oxygen partial pressure. It is, therefore, important to determine how DDMPS is retained in the PDMS matrix. We tested DDMPS retention in the PDMS matrix by examining the weight change of devices over time. The weights of devices at 0, 2, and 4 weeks were recorded and compared to detect any loss of DDMPS (Figure 4.4). Devices immersed in deionized water and fetal bovine serum (FBS) at 37°C for over a month showed less than 1% weight change, whereas DDMPS gradually escapes from devices exposed to air. The lack of weight loss for sensors placed in an aqueous environment can be attributed to the low solubility of siloxanes in those solutions. The aqueous solution, in essence, creates a barrier for transport of low molecular weight siloxanes into the headspace of the container. Although it is not possible to quantify the amount of siloxane retained in vivo, this result suggests that the siloxane will be retained long-term in the body.
Figure 4.4: DDMPS does not partition into either deionized (DI) water or fetal bovine serum (FBS). DDMPS can, however, freely diffuse in and out of the sensor, as evidenced by the weight loss experienced by sensors placed in air. The lack of weight loss for sensors placed in an aqueous environment can be attributed to the low solubility of siloxanes in those solutions. Thus, the aqueous solution creates a barrier for transport of low molecular weight siloxanes into the headspace of the container. The favorable partition coefficient is a likely factor that keeps the materials functionally stable in the body. Reference: Liu, V.H., Vassiliou, C.C., Imaad, S.M. & Cima, M.J. Solid MRI contrast agents for long-term, quantitative in vivo oxygen sensing. *Proceedings of the National Academy of Sciences* (2014).

All implantable devices must be sterilized prior to use. An improperly sterilized device can lead to infections at the implantation site and even device failure as a result of host response to foreign antigens. Autoclaving is one of the most commonly used sterilization techniques for medical instruments. Autoclaves sterilize their contents by exposing them to heated steam at 121°C for 20 minutes. Autoclaving is reliable, does not require the use of harmful chemicals, and sterilizes devices of different formats including those immersed in solution. The temperatures used in the autoclaving process would nominally promote volatile siloxanes to escape and cause performance degradation in the sensor, but immersing the devices in an aqueous environment prevented siloxanes from escaping and precluded changes in device performance (Figure 4.5). An energy
barrier created by the aqueous solution, similar to that described in the previous section, prevents siloxane escape during the autoclaving process and maintains device stability.

Figure 4.5: DDMPS/PDMS Devices autoclaved while immersed in water retains its performance characteristics compared to devices autoclaved in air. The aqueous environment prevents siloxane escape by creating a barrier that prevents transport of siloxanes into the headspace of the container. Reference: Liu, V.H., Vassiliou, C.C., Imaad, S.M. & Cima, M.J. Solid MRI contrast agents for long-term, quantitative in vivo oxygen sensing. Proceedings of the National Academy of Sciences (2014).
4.3 MRI Studies

We measured the longitudinal relaxation times of 70% DDMPS/PDMS devices in a Varian 7T small animal magnetic resonance imager (MRI). An inversion recovery pulse sequence with exponentially spaced points along the longitudinal relaxation curve was used to measure $T_1$ in the MRI. This translates to capturing an image at each inversion time point and extracting the intensity of particular regions of interest at each of those points (Figure 4.6). The extracted intensities can then be mapped to the inversion recovery equation. Intensity values by definition are non-negative values, meaning that the inversion recovery equation needs to be modified to $I = I_0 (1 - f_{inv} e^{-\frac{t}{\bar{T}_1}})$ where $f_{inv}$ represents the effectively inverted proton fraction$^{12}$. The absolute value function in the equation allows the non-negative values to be mapped properly to the inversion recovery equation. A representative data curve is shown in Figure 4.7. MRI data acquisition was done with the following parameters: matrix size = 64x64, FoV = 32mm x 32mm, slice thickness = 2mm, TE = 12.5ms, ETL = 16, and kzero = 8. The effective TE of sequence used was 100ms. TR was adjusted depending on the $T_1$ of particular samples and was set to at least 3x measured $T_1$. Eight inversion times, separated exponentially, were used to generate data for $T_1$ mapping.
Figure 4.6: MRI scan of 4 different siloxanes embedded in PDMS at 70% weight/weight. The siloxanes were contained in glass vials, which are placed in a bottle with DI water to minimize susceptibility artifacts. The temperature was kept constant at 37°C with the use of a forced hot air circulator. A fast spin-echo pulse sequence with inversion recovery was used to generate the images at 8 different inversion times; intensity in each of the image can then be used for extracting the $T_1$ relaxation time.
DDMPS and PDMS retained distinct relaxation characteristics in the devices. The measured signal from these devices is a sum of the signals from PDMS and DDMPS. The inversion recovery equation can be expanded to describe data obtained from a mixture of two materials:

$$I = f I_o \left(1 - 2e^{-\frac{t}{T_{1,DDMPS}}}\right) e^{-\frac{TE}{T_{2,DDMPS}}} + (1 - f) I_o \left(1 - 2e^{-\frac{t}{T_{1,PDMS}}}\right) e^{-\frac{TE}{T_{2,PDMS}}}$$

where $f$ denotes the fraction of DDMPS in the mixture while $1 - f$ denotes the fraction of PDMS in the mixture. If TE is small compared to the transverse relaxation time of both materials, a double exponential behavior is observed (Figure 4.8). Two distinct relaxation times can be extracted by fitting the relaxation data to the equation described above. Accurately fitting data to
equations with multiple variables is difficult since there are many degrees of freedom with which the equation can be fitted.

Figure 4.8: 70% DDMPS/PDMS devices exhibit double-exponential relaxation behavior when the data is acquired at low TE (0.5ms). The data contained signal from both DDMPS and PDMS, and fitting to the appropriate model is required to extract the correct relaxation times.

Signal from the slower relaxing component dominates the overall acquired signal if TE is long compared to the transverse relaxation time of the fast relaxing component. When data is acquired using a long TE, signal from the fast relaxing component has completely relaxed, allowing us to simplify the equation:

\[ I = f I_o \left( 1 - 2e^{\frac{-t}{T_{1,DDMPS}}} \right) e^{\frac{-TE}{T_{2,DDMPS}}} \]
Using a long echo time to differentiate the signals has several advantages over spectroscopic methods, a common approach used to isolate signals in MRI. A method based on relaxation time is easier to implement and can be used to separate otherwise similar materials. PDMS and DDMPS, for example, both have chemical shifts of roughly 4ppm from water, which translates to about 1.2 kHz at 7 Tesla. A highly uniform sample and well-shimmed magnetic field are needed to separate the signals by spectroscopy since the difference in chemical shift between PDMS and DDMPS is very small. This is difficult to achieve since many elements introduce susceptibility differences in the body.

PDMS has a short $T_2$ as shown in Figure 4.9, and its contribution to the overall signal becomes negligible at echo times of 100ms or longer. Using a long echo time of 100ms effectively reduces the signal intensity of PDMS to less than 20% of its initial value. The signal from PDMS should constitute less than 1% of the overall measured signal since PDMS already started at a lower intensity value. The relaxation data of 70% DDMPS in PDMS exhibits a mono-exponential behavior (Figure 4.10) and the acquired relaxation time is comparable to neat DDMPS. Figure 4.11 shows the $T_1$ of neat siloxanes compared to siloxanes incorporated in PDMS at 70%, with the data acquired at an echo time of 100ms. It can be seen that the longitudinal relaxation rates of the two sample sets are the same. These results suggest that the two siloxanes have limited interactions between them and confirmed that the longitudinal relaxation times are only minimally affected by the matrix.
Figure 4.9: MRI scans of phantoms containing different siloxanes. The phantoms were in glass tubes and are immersed in a container filled with DI water to minimize magnetic susceptibility differences. PDMS has a $T_2$ of 20 ms at 7T, and the average intensity of PDMS phantom drops to less than 20% of its initial value at an echo time of 100ms. Coupled with a lower initial intensity value, signal from PDMS would constitute less than 1% of acquired signal at 100ms. 70% DDMPS 30% PDMS phantoms have lower intensity values throughout the measurement period due to an effectively smaller amount of sample but the extracted $T_2$ value is not significantly different from pure DDMPS. Insert: Representative image of phantoms acquired using an echo time of 100ms. The image is obtained from an axial slice, and shows a cross-sectional view of the phantoms. Top: PDMS. Bottom left: DDMPS. Bottom right: 70% DDMPS 30% PDMS. Reference: Liu, V.H., Vassiliou, C.C., Imaad, S.M. & Cima, M.J. Solid MRI contrast agents for long-term, quantitative in vivo oxygen sensing. *Proceedings of the National Academy of Sciences* (2014).
Figure 4.10: Normalized relaxation time of DDMPS and 70%DDMPS measured using the MRI at an echo time (TE) of 100ms. The averaged relaxation times for DDMPS and 70% DDMPS are $2.6 \pm 0.06$ s and $2.55 \pm 0.12$ s, respectively. The longitudinal relaxation time of DDMPS (solvent) dominates the overall signal at high echo times. Additionally, the relaxation data is well fitted by a mono-exponential fitting scheme.
Figure 4.11: Comparison between neat siloxane and siloxane incorporated into PDMS at 70 w/w% in air (n = 3). The relaxation rates of neat siloxane material are not significantly different from material incorporated into PDMS, indicating that signal from the PDMS matrix can be effectively suppressed. The data points are based on 6 measurements in the MRI at 37 °C and error bars represent standard deviations of measurements. Reference: Liu, V.H., Vassiliou, C.C., Imaad, S.M. & Cima, M.J. Solid MRI contrast agents for long-term, quantitative in vivo oxygen sensing. Proceedings of the National Academy of Sciences (2014).

The value of transverse relaxation time is determined by molecular motion in the material. The relationship between molecular structure and molecular motion have on transverse relaxation time is well studied in polymer systems: the crystalline regions in a polymer, for example, have transverse relaxation times in the microseconds range, whereas the amorphous regions of the same polymer have transverse relaxation times in the milliseconds to seconds range. Factors that affect molecular mobility, such as the presence of cross-links and physical polymer chain entanglement, have a proportional effect on the transverse relaxation time. The impact of crosslinks and physical entanglements primarily affects the transverse relaxation time, even though the longitudinal relaxation times also vary with
molecular motion. Comparing the relaxation times of uncrosslinked Sylgard 184 PDMS to crosslinked Sylgard 184 PDMS, it can be observed that the $T_1$ changed from 550 ms to 480 ms while the $T_2$ decreased from 115 ms to 25 ms (Figure 4.12). $T_2$ is heavily affected by physical interaction, whereas $T_1$ is largely unaffected because $T_1$ relaxation is dominated by high frequency molecular tumbling\textsuperscript{15}. The measured $T_1$ of materials in a mixture would thus have a similar longitudinal relaxation time as neat material.

![Graph of relaxation times](image1)

**Figure 4.12:** Evolution of Sylgard 184 PDMS relaxation times as curing occurs. The $T_1$ of PDMS decreased by around 13\% (from 550ms to 480ms) while the $T_2$ decreased by 78\% (from 114ms to 25ms) as the polymer turns from liquid into solid.

The response kinetics and dynamic range of 70\% DDMPS/PDMS devices was characterized in the MRI with intensity measurements. Cylindrical devices of different sizes were exposed to step changes in oxygen partial pressure. Intensity
measurements were used as surrogate markers for $T_1$ because of the need to capture fast kinetics, and the inversion time chosen for this experiment was designed to maximize the intensity difference between the various oxygen partial pressures. Steady state was achieved in these devices in less than 10 minutes after a change in oxygen partial pressure, suggesting that the PDMS/siloxane devices have similar kinetics as neat siloxane (Figure 4.13).

![Figure 4.13: Kinetics of response of 70% DDMPS 30%PDMS phantoms are similar to liquid DDMPS. These phantoms were exposed to changing oxygen partial pressure in the MRI, and image intensity was used as a surrogate indicator of $T_1$. Images were taken every 2 minutes with a coronal orientation and the intensity in the phantoms was averaged to estimate time to steady state. We found that steady state was achieved in less than 10 minutes for phantoms of all sizes. Reference: Liu, V.H., Vassiliou, C.C., Imaad, S.M. & Cima, M.J. Solid MRI contrast agents for long-term, quantitative in vivo oxygen sensing. *Proceedings of the National Academy of Sciences* (2014).](image)

The relaxation rates of 70%DDMPS/PDMS devices were additionally characterized at three different temperatures. The temperature dependence of $T_1$ stems from the variation of correlation time with temperature. Correlation time is related to molecular motion and can be considered a molecular-scale viscosity. The energy of molecules and associated molecular motion increases with temperature which leads to a more rapid dipolar field fluctuation. Our materials have short correlation times (evidenced by the long relaxation time), and $T_1$ is
positively correlated with sample temperature\textsuperscript{16}. The temperature range of interest is close to the normal physiological value of 37°C, and we measured the response at 25, 31, and 37°C to examine the effects of temperature.

The relaxation rates (reciprocal of relaxation time) and temperature have a negative correlation, as expected (Figure 4.14). The effect temperature has on relaxation time is comparable to the measurement error if the temperature fluctuation is small (less than 1 degree centigrade) (Table 4.1), and temperature is not expected to greatly affect the accuracy of our results. A method for quantifying temperature variations, however, can improve measurement accuracy for applications involving larger temperature changes. An example of such an application would be the thermal therapy of diseases. Several MR parameters are temperature-sensitive and can be used to measure the temperature of the surrounding tissue and injected sensors. These methods, such as diffusion measurements and shifts in resonance frequency\textsuperscript{17}, can be used as correction factors and allow the measured relaxation time to be interpreted in the correct context.
Figure 4.14: Relaxation rates of 70% DDMPS/PDMS phantoms were measured in the MRI at 3 different temperatures (n=3). Error bars denote standard deviations. The measured relaxation rate was found to have a negative correlation with temperature at all oxygen partial pressures. Reference: Liu, V.H., Vassiliou, C.C., Imaad, S.M. & Cima, M.J. Solid MRI contrast agents for long-term, quantitative in vivo oxygen sensing. Proceedings of the National Academy of Sciences (2014).

<table>
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<th>Oxygen partial pressure (%)</th>
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</tr>
<tr>
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</tbody>
</table>

Table 4.1: The error introduced by temperature variations is comparable to measurement deviations.
4.4 DDMPS/PDMS Microparticles

While PDMS/DDMPS devices can be molded into different sizes easily, devices smaller than 1.2 mm (16 gauge) are difficult to handle due to the elastomeric nature of the material. The devices can flex and warp when being pushed out of a needle by a plunger. Needles with large bores or long devices would be needed if a large sensitive volume were required. This issue was solved with the use of an ensemble of smaller devices that self-assemble into a larger sensor. Small PDMS/DDMPS sensors can be synthesized in a microparticulate formulation by emulsification. Siloxanes are highly hydrophobic and immiscible with water, and microparticles can be created as an oil-in-water emulsion. The PDMS/siloxane base solution was first added to a deionized (DI) water solution of 5wt% Vitamin E-TPGS (Sigma-Aldrich, USA). The final mixture consists of 30% PDMS/siloxane with a total volume of 10mL. This mixture was then subjected to high shear forces with a tissue homogenizer (Silverson Machines, USA) to form a suspension of PDMS/siloxane microparticles. The homogenizer was run at 5000 rpm for 4 minutes with a high shear screen and a square-hole work head. The resulting emulsion was then heat-cured under stirring in a water bath at 65°C overnight to form solid microparticles.

The particles were washed three times with deionized (DI) water to remove the excess surfactants. The particles were first centrifuged at 2000 rpm for 5 minutes. The supernatant was then removed with a Pasteur pipette. DI water is then added back to the container at a 10:1 volume ratio of DI water: particles and then vigorously mixed with the particles. The particles were re-suspended in sterile saline prior to injection.

We designed these particles to assemble into a depot once they are injected. The primary mechanism of depot formation is filtration of saline from the particles.
suspension. The PDMS particles suspended in solution are separated by thin layers of water and stabilized by surfactants; the barrier between these particles would collapse when the water is resorbed by the body, allowing the particles to fuse together at the site of injection (Figure 4.15). The particles would thus coalesce into a single sensor once the saline has filtered into the surrounding tissue. Most tissue has sufficient connective matrix to act as an appropriate filter, but these sensors are not suitable for injection into arterial, venous, or cerebrospinal fluid. This physical interaction allows the fused device to remain intact for one month, even though this fusing does not create a new chemically crosslinked entity. An explanted muscle tissue shows that the particles have fused together into a single depot that resisted displacement during normal animal movement (Figure 4.16). The depots of contrast agent formed reliably in different animals, but the exact shape or size of the depots differed between animals because these physical parameters were altered by the specific tissue structures and how quickly the saline was cleared.

Figure 4.15: The microparticles are synthesized in a size range that would enable their retention in most tissue types. Once the particle suspension is injected, the filtering of saline into the surrounding tissue allows the particles to come into contact with each other. The non-specific interaction of PDMS allows the particles to coalesce into a single depot sensor.
Figure 4.16: An explanted piece of rat muscle tissue with injected microparticles shows the fusion of particles in the body. The particles resided inside the animal for 4 weeks and resisted displacement from normal animal movement.

We found that microparticles measuring around 15 microns worked well in maximizing flow in the injection process. Particles of this size are large enough to avoid clearance but small enough to be easily injectable via a 29-gauge needle. Particle size is determined by the concentration of surfactants, the ratio between oil phase and aqueous phase, and the spin speed of the tissue homogenizer. Qualitatively, the particle size decreases with increases in spin speed, increases in process time, and increases in surfactant concentration. The particle size decreases with decreased ratio of oil phase to aqueous phase. These findings are summarized in Figure 4.17.

The longitudinal relaxation time of microparticles was compared to that of molded devices to ensure that this different formulation and processing does not result in unexpected changes in relaxation time. Relaxation times were measured at various oxygen partial pressures using the Bruker Minispec spectrometer. Measuring the microparticles in the minispec necessitates the water contribution to be minimized as water would introduce a different relaxation component in the signal. This was achieved by re-suspending the particles in deuterated water before measurement. Deuterated water has a different resonant frequency and does not contribute to the signal. We found the response of microparticles to be similar to that of molded
devices (Figure 4.18) and confirmed that this material can be processed in different manners without altering the microscopic physical properties. The microparticles could be stored in DI water without degradation in performance. A 5% solution of surfactants should be used in the solution, if an extended storage period is needed, to prevent agglomeration of the microparticles.

4.5 Conclusion
The solid contrast agents provided a way to accomplish long-term monitoring of oxygen tension. While this paper demonstrated a solid contrast agent for measuring oxygen tension, the same concept can be extended for use with other responsive contrast agents. Two design criteria need to be satisfied for successful implementation of this concept. First, a matrix that retains the contrast agents (i.e. with favorable partition coefficient) and is permeable to the analyte of interest is required. The second criterion involves a mechanism for contrast generation. Oxygen molecules are inherently paramagnetic and increase relaxation rate of surrounding molecules, but other responsive contrast agents can be used with analytes that are nonmagnetic to enable their detection.
Figure 4.17: The impact of process parameters on particle size. One of the process parameters is varied on each row while the rest were held to be the same. The particles were cured using the same method and the washing steps were the same across different samples.
Figure 4.18: The longitudinal relaxation times of the microparticles are similar to the relaxation times measured with larger devices.
References


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Chapter 5

Oxygen Sensing in Animal Models

This chapter describes *in vivo* measurements performed in rodent models with Magnetic Resonance Imaging (MRI). Two sets of experiments are used to highlight the use of these sensors and how they can apply to clinical situations. The first set of experiment establishes that these sensors can detect changes of oxygen in the body for one month or longer, and validates the concept of contrast agent retention in a polymeric matrix. The second set of experiment examined oxygen response in situations where blood flow has been restricted by trauma or other ischemic diseases.

5.1 Signal isolation in the MRI

The smallest image element in magnetic resonance imaging (MRI) is the voxel, representing an intensity value in 3-dimensional space. Voxels are generated through the application of magnetic field gradients, which mark each spatial coordinate with an offset in resonant frequency. Signals received at different frequencies are then deconvoluted into an image using the inverse Fourier transform. The size of these voxels is determined not by the imaging equipment, but by tradeoffs involving scan time and signal-to-noise ratio (SNR). Smaller voxels contain proportionally lesser amount of signal and require more signal averaging to achieve the same SNR. This necessitates a longer scan time. A high spatial resolution is necessary to avoid the partial volume effect, a situation where different tissue components overlap in the same voxel. The affected voxels would, for example, contain signal from both the implanted device and surrounding...
tissue. The relaxation data extracted from these voxels would contain two exponentials because two distinct components are present.

An alternative method to prevent the overlapping of signals in a voxel is selective excitation. We exploited the difference in transverse relaxation time of tissue and our DDMPS/PDMS devices to selectively acquire signal from the injected sensor devices. Muscle tissue has a short transverse relaxation time ($T_2$) that can be suppressed with a spin-echo sequence with long echo time (Figure 5.1), similarly to PDMS. The device can be reliably distinguished from the background once signal from the muscle tissue is suppressed. The signal in each voxel consists predominately of signal from the device because the muscle signal has significantly decayed at the acquisition time. The intensity of these voxels acquired at different inversion times could then be mapped to the inversion recovery equation to obtain a map of $T_1$ values.
Figure 5.1: Tissue background signal can be suppressed with the use of long echo times in a spin-echo sequence. The intensity of muscle signal can be seen to significantly decrease when the echo time was increased from 10ms (left) to 100ms (right). Both the implanted device (top) and injected microparticles (bottom) retained a relatively high intensity even at a high TE.

5.2 Inspired gas experiments

Validation with animal models is a necessary step to verify the functionalities of implantable devices. This is especially important for devices designed to monitor changes \textit{in vivo}, since many \textit{in vitro} models oversimplify environments encountered in the body. The response and kinetics of sensors could be impaired in the body over time due to bio-fouling, sensor migration, and host tissue response\textsuperscript{1}. Previous studies have reported alteration of oxygen sensitivity in sensing materials within days or weeks of implantation\textsuperscript{2,3}. Factors leading to loss of oxygen sensitivity include chemical modification of the sensor, changes in material properties, and modification of the physical environment surrounding the sensors\textsuperscript{4}. There are thus two reasons to test these sensors in an animal model: to investigate sensor response to changes in tissue oxygen tension, and to ascertain consistency of measurement performance over time.
Inspired gas change is a reliable method for establishing different oxygen tensions \textit{in vivo} and creating reference points for comparing studies conducted at different tiempoints\textsuperscript{1}. Clinical research has suggested that hyperbaric oxygen therapy can alleviate hypoxia encountered in solid tumors. The use of oxygen therapy in these scenarios can benefit from knowledge of oxygen partial pressure\textsuperscript{5,6}. We are using inspired gas change as a method to study the functional stability of these materials, as any instability \textit{in vivo} would manifest as a baseline drift in sensor readings. Since the animal physiology should only be minimally perturbed by changes in inspired gas, sensor baseline should only change minimally over time. Readings from a pulse oximeter, clipped to the rat’s toe, were used as an external standard to track any changes in animal physiology between experiments.

All animal experiments were conducted in accordance with the guidelines of the Committee on Animal Care (CAC) at MIT. The rats were anesthetized using 2\% isoflurane, and 30 µL of DDMPS/PDMS microparticles suspended in sterile saline were injected into the calf muscle of each rat. This particles suspension has a solid fraction of roughly 66\% v/v. The microparticles aggregated into a depot that became a single sensor once injected. Since approximately 20µL of solid material was present in the depot, the equivalent spherical diameter was 1.68mm. The rats were placed into the MRI under anesthesia after contrast agent injection. Each rat was imaged for 1.5 hours with the experiment divided into three 30-minute segments: a baseline measurement for the first 30 minutes with the rat breathing oxygen, then an experimental measurement for 30 minutes with the rat breathing medical air, and finally a recovery measurement for 30 minutes with the rat breathing oxygen. MRI Measurements were performed every five minutes, with the rat’s breathing and heart rate monitored by a SA Instruments small animal monitoring system throughout the experiment. Animal body temperature was maintained at 37\°C with a MR-compatible forced hot air circulator.
Oxygen partial pressure of the injected depot of particles was obtained by mapping the average intensities of pixels within the sensor area to the inversion recovery equation. A long echo time was used to ensure single exponential behavior in the data. The fitted $T_1$ value was then converted to oxygen partial pressure with the calibration curve for DDMPS/PDMS. We found that steady state readings for each sensor were registered in less than 15 minutes and the response was reversible. Interestingly, the measured oxygen tensions were different between animals breathing the same inspired gas. This phenomenon is likely due to a combination of the idiosyncratic physiology of the animals and variations in injection location of the microparticles. Microparticles that are located in better-perfused areas, for example, would register a higher oxygen tension compared to particles injected in a poorly perfused area. We envision that making these injections under imaging guidance should help equalize the variations observed here.

An external standard was needed to correlate results from different studies because of the aforementioned variations between animals. An independent oxygen tension measurement was needed to establish this external standard. Pulse oximetry is a widely accepted method for measuring oxygen tension in the vasculature and was chosen as the independent measurement method. While pulse oximetry does not provide the oxygen tension at the same locations as the injected sensors, changes in systemic oxygen tension would be reflected in the pulse oximetry readings. A linear regression line was used to relate pulse oximeter readings to MR sensor measurements. The extracted oxygen tensions at both week 0 and week 4 lie in the same range (Figure 5.2), suggesting that microparticles injected for a period of 4 weeks had minimal impact on oxygen tension at the host tissue site. The kinetics of response was unaffected and the time to steady state was similar between measurements taken at 0 and 4 weeks. The calculated slope of the linear regression line was used to quantitatively relate the oximeter and microparticle sensor
readings. The calculated slope is significantly similar between measurements taken at 0 and 4 weeks. While the specific oxygen tension in a particular animal could be different between measurements, the similar correlation of the slopes suggests that the sensor performance was not impaired for the one month long implantation duration. Images of injected sensors can be overlaid on anatomical features to provide better context for diagnostic purposes (Figure 5.3).

The microparticles resisted migration once they were injected. The particles fused to form a solid depot in between muscle fibers upon injection, which prevented individual particles from being cleared by the body. This depot could thus be considered a solid device and conforms to surrounding tissue. PDMS is a biocompatible material and we observed only a thin layer of fibrous capsule (~25 μm) around the devices (Figure 5.4). The diffusion of oxygen was not impeded by the formation of this thin layer of scar tissue and the sensors remained stable for an extended period of time.
Figure 5.2: Oxygen measurements extracted from sensors immediately after injection (top) and 4 weeks after injection (bottom) are plotted against pulse oximeter measurements (n = 4). Each data point represents an average of 6 measurements in each animal and error bars denote standard deviations of measurements. A linear regression line is used to find the correlation between sensor measurements and pulse oximeter readings. The values of the regression slopes are found to be similar in both measurements, indicating similar correlation between pulse oximeter readings and sensor measurements over the 4-week period. Deviations from the line are likely due to variations in local tissue oxygen level and differences in injection location. Blue: oxygen (initial); red: air; cyan: oxygen (reverse). Reference: Liu, V.H., Vassiliou, C.C., Imaad, S.M. & Cima, M.J. Solid MRI contrast agents for long-term, quantitative in vivo oxygen sensing. Proceedings of the National Academy of Sciences (2014).
Figure 5.3: Maps of extracted oxygen tension can be overlaid on anatomical images to provide both physical and biochemical information. These two representative MRI images are coronal slices of the leg and are taken from animals breathing oxygen (left) and air (right) respectively. Reference: Liu, V.H., Vassiliou, C.C., Imaad, S.M. & Cima, M.J. Solid MRI contrast agents for long-term, quantitative in vivo oxygen sensing. *Proceedings of the National Academy of Sciences* (2014).

Figure 5.4: H&E stained micrographs showed that the 70% DDMPS/PDMS microparticles fused into a depot that resisted migration after injection. There is minimal foreign body reaction to the injected microparticles as evidenced by the thin fibrous capsule surrounding the injection site. Reference: Liu, V.H., Vassiliou, C.C., Imaad, S.M. & Cima, M.J. Solid MRI contrast agents for long-term, quantitative in vivo oxygen sensing. *Proceedings of the National Academy of Sciences* (2014).
5.3 Circulation Restriction Experiments

Compartment syndrome is a serious complication caused by acute damage to the extremity and subsequent swelling in tissue\textsuperscript{9}. Compartment syndrome is typically treated with fasciotomy, an invasive procedure where the fascia is cut to relieve the pressure. Less invasive treatments are available but an assessment of tissue oxygen tension is needed to determine the severity of the condition. We modeled compartment syndrome by modulating the pressure of a nylon pressure cuff placed around the thigh of a Sprague-Dawley rat. This model tested the functionality of the injected sensors with changes in blood flow and when subjected to movement and physical stress. The inflation of this cuff directly compresses the tissue underneath, increases compartmental pressure, and restricts blood flow to the tissue downstream.

Tissue oxygen measurements were made using an injected bolus of 70\% DDMPS/PDMS microparticles in the MRI. The microparticles were injected into the calf muscle of the rats prior to measurements, and a step-wise increase of pressure level was used to simulate increasing levels of compressive injury. The pressure cuff was placed proximal to the injection site on the rat’s leg and inflated after a baseline measurement was taken. The cuff pressure was changed in a stepwise pattern: first increasing from 0 mmHg to 75 mmHg, and finally to 150 mmHg. Similar to the inspired gas experiments a pulse oximeter on the rat’s toe provided an independent oxygen tension measurement. Examples from literature have shown transcutaneous oxygen tension measurements to be susceptible to factors such as local blood flow and arterial oxygen content\textsuperscript{10,11}, and we found that the pulse oximeter readings became unstable when circulation was restricted. The contrast agents described in this paper directly detected the decreased oxygen tension caused by the increased compartment pressure (Figure 5.5) and were unaffected by changes in blood flow. The depot of contrast agents was not
damaged or displaced when the compartmental pressure increased, making the sensor suitable for use in locations where movement or physical stress is expected.

Figure 5.5: The injected contrast agents resisted physical stress and continued functioning irrespective of blood flow. Oxygen tension extracted during circulation restriction experiment (n=3). Error bars denote standard deviations across different animals. Pulse oximeter readings become unavailable when the pressure was raised to 75 mmHg and 150 mmHg pressure levels and are not presented here. The oxygen level was observed to return to the initial level after the pressure cuff was released. Representative pixel maps of extracted oxygen tension are overlaid with MRI scans of the animal at different cuff pressure levels. The pictures are coronal slices of the animal’s leg. Sensor migration, even at the highest pressure level applied, was not observed. Reference: Liu, V.H., Vassiliou, C.C., Imaad, S.M. & Cima, M.J. Solid MRI contrast agents for long-term, quantitative in vivo oxygen sensing. Proceedings of the National Academy of Sciences (2014).
5.4 Data Analysis

Voxels containing the contrast agents were isolated with specific MRI pulse sequence parameters and a custom MATLAB script. The pulse sequence created an intensity difference between the device and tissue in the images, which allowed an automated MATLAB script to identify the pixels containing the contrast agent through intensity differences. This automatic selection was done in the context of a user-selected area of interest. Pixels that didn’t fit well to the inversion recovery model were discarded, as the data in these pixels was deemed too noisy to be useful.

The data was analyzed in two different ways once the relevant pixels were selected. The first approach treats the injected contrast agents as a single sensor. Intensity was averaged across the selected pixels and fitted to the Inversion recovery equation

\[ I = I_o \left( 1 - f_{inv} e^{-\frac{t}{T_1}} \right) \]

using MATLAB to obtain the longitudinal relaxation time. \( f_{inv} \) represented the effective fraction of inverted protons and accounts for equipment or pulse sequence imperfections\(^\text{12}\). Relaxation times were then converted to oxygen partial pressure with a calibration curve. The second approach treated each pixel as an individual sensor. The intensity of each pixel was mapped to the inversion recovery equation to obtain the longitudinal relaxation time for that pixel. A \( T_1 \) map resulting from this analysis can then be converted into a map of oxygen partial pressure with a calibration curve. The map of oxygen tension can be overlaid on anatomical features to relate oxygen partial pressure to tissue structures. This process is outlined in Figure 5.6, below, to demonstrate the data analysis process.
Figure 5.6: After signal from the matrix material and muscle tissue background are suppressed with appropriately chosen pulse sequence parameters, unwanted signal is excluded with proper ROI selection. An automated script then calculates the relaxation times and extracts the metabolite concentrations.

5.5 Conclusions

*In vivo* monitoring of oxygen tension with solid contrast agents was demonstrated. These injectable solid contrast agents allow for monitoring of specific tissue sites independently of blood flow compared to other magnetic resonance based approaches. These contrast agents can thus be used to monitor regions with poor perfusion or conditions involving compromised blood flow. We have additionally demonstrated that these sensors would stay functional in the same tissue site for at least four weeks; this allows for longitudinal imaging to monitor the same tissue site and eliminate possible confusion from spatial or temporal variations of contrast agent concentration.

5.6 Materials and Methods

**Microparticle Synthesis**

Microparticles are created as an oil-in-water emulsion. The PDMS/siloxane base solution was first added to a deionized (DI) water solution of 5w/w percent Vitamin E-TPGS (Sigma-Aldrich, USA). The final mixture consists of 30% PDMS/siloxane with a total volume of 10mL. This mixture was then subjected to high shear forces with a tissue homogenizer (Silverson Machines, USA) to form a
suspension of PDMS/siloxane microparticles. The homogenizer was run at 5000 rpm for 4 minutes with a high shear screen and a square-hole work head. The resulting emulsion was then heat-cured under stirring in a water bath at 65°C overnight to form solid microparticles.

The particles were washed three times with deionized (DI) water to remove the excess surfactants. The particles were first centrifuged at 2000 rpm for 5 minutes. The supernatant was then removed with a Pasteur pipette. DI water is then added back to the container at a 10:1 volume ratio of DI water : particles and then vigorously mixed with the particles. The particles were re-suspended in sterile saline prior to injection.

**Magnetic Resonance Imaging**
Magnetic Resonance Imaging was performed using a 7 T small animal imager (Agilent, USA). The imaging protocol includes a scout pulse sequence for locating devices and a fast spin-echo sequence with inversion recovery for performing $T_1$ measurements with the following parameters: matrix size = 64 x 64, FoV = 32 mm x 32 mm, slice thickness = 2 mm, TE = 12.5 ms, ETL = 16, and kzero = 8. The effective TE of sequence used was 100 ms. TR was adjusted depending on the $T_1$ of particular samples and was set to at least 3x measured $T_1$. Eight inversion times, separated exponentially, were used to generate data for $T_1$ mapping. The measurements were performed in a quadrature volume coil that measures 63mm in diameter. The excitation pulse and refocusing pulses had a 3 kHz bandwidth, while the receiver bandwidth was 100 kHz. No pre-saturation pulses were applied for these studies.

**Histology**
Histology samples were prepared by immersing excised tissue in 4% Paraformaldehyde solution in PBS overnight. The samples were then transferred
to and stored in a 70% Ethanol solution until they were further processed. H&E staining was performed by the Histology Core facility at the Koch Institute for Integrative Cancer Research.
References


Chapter 6

MR Readable pH sensors

This chapter describes a novel technology for detecting pH changes in the body using magnetic resonance technology. pH is an important indicator of tissue health and pH homeostasis is tightly regulated using various buffering systems in the body. pH deregulation is associated with several pathologies, with cancer being the most prominent example. Unlike the work described in the other chapters, we synthesized these pH-sensing materials for use with an implantable micro-resonator device designed to perform single voxel pH detection. This work was performed in collaboration with Christophoros C. Vassiliou.

6.1 Motivation

The pH in biological environment regulates the function of many enzymes and is integral in maintaining the structure of proteins. Significant fluctuations of pH levels, particularly in blood, can be life threatening. Cancer is perhaps the best-studied example where alterations in pH featured importantly in disease progression and treatment. An acidified interstitial pH is a hallmark of cancer, where the reduced buffering capacity and increased lactic acid production result in an acidified environment\(^1\). This acidified tumor microenvironment produces many biological changes that inhibit chemotherapy and radiotherapy\(^2\), and a new class of chemotherapeutics is now being developed to specifically target the acidic tumor microenvironment\(^3\). Precise knowledge of local pH at the tumor site can be used to verify the efficacy of these drugs and stratify patient response. Intratumoural, extracellular pH has recently been used as a biomarker for tumor response to chemotherapy by Lindner and Raghavan\(^4\). Their study showed a differential pH
response between tumors arising from a drug-sensitive cell line and a drug-resistant cell line. The extracellular pH in drug-sensitive tumors decreased after administration of Doxorubicin, a chemotherapeutic. Tumors resistant to Doxorubicin, conversely, did not exhibit the same changes (Figure 6.1). Since many chemotherapeutics are toxic and carry significant side effects, a technology that speeds detection of treatment response would help minimize these toxic side effects in patients. Tissue pH changes occur only locally and cannot be measured using systemic measurement approaches. The ideal measurement technology would also provide pH measurements throughout the entire treatment duration so that the treatment can be properly tailored.

Figure 6.1: Extracellular, intratumoural pH is depressed from metabolic changes as a result of the irregular vasculature network found in a tumor. Tumors responding to a chemotherapeutic were found to have a pH value further depressed to pH 5.5, whereas the pH in tumors resistant to the chemotherapeutic remains unchanged. Reference: D. Linder and D. Raghavan. Br J Cancer (2009), 100, p1287

6.2 Existing technologies

The current gold standard for measuring local tissue pH is the electrochemical probe. pH readings from these probes are obtained by measuring the difference in electrochemical potential between a sensing electrode and a reference electrode. This difference in electrochemical potential generates a voltage that can be measured and converted to pH with a calibration curve. pH electrodes are fast and
accurate but are invasive and impractical for frequent measurements in the body. Implantable electrodes are available but their measurements are unreliable due to baseline drift and biofouling on the sensing electrode. These characteristics make the pH electrode impractical for long-term measurements.

6.3 MR Technologies
Non-invasive imaging offers an attractive alternative strategy for measuring local tissue pH. Several MR spectroscopy and MR imaging methods were developed for measuring pH; these methods can be further divided into those that worked with endogenous metabolites and those that required administration of contrast agents. An example of a currently used spectroscopic method for measuring intracellular pH is $^{31}$P MR spectroscopy. This method measures the signal of inorganic phosphate molecules, such as those found on ATP, and infers pH from that information. Signals from exogenous probes, such as 3-aminopropyl-lphosphate (3-APP) and 3-[$N$-(4-fluor-2-trifluoromethylphenyl)-sulphamoyl]-propionic acid, have been used by some researchers to measure extracellular pH in cancer studies with $^{31}$P and $^{19}$F spectroscopy, respectively. Proton MR spectroscopy has also been used for pH measurements with an exogenously administered imidazole, IEPA.

Non-spectroscopic methods for measuring pH include the use of pH-sensitive Gadolinium (Gd) complexes and pH-dependent chemical exchange. pH-sensitive Gd complexes allows pH to be imaged with high spatial resolution, but the concentration-dependent nature of these contrast agents makes accurate determination of pH values difficult. The fast clearance rate of these contrast agents is also detrimental to long-term monitoring applications. Chemical exchange contrast, in comparison, is found on both polymers and small molecules in solution. Large polymer molecules can be built to withstand systemic clearance and be used as a long-term contrast agent for pH.
Chemical exchange saturation transfer (CEST) contrast is observed in systems with multiple water protons compartments. Water protons experience different rates of molecular motion in each of these compartments and exhibit different relaxation behavior. Water absorbed onto the surface of polymers, for example, has significantly shortened relaxation times compared to free water due to interaction with the polymer protons. These compartments may also have different resonant frequencies in addition to different relaxation behavior\textsuperscript{11}. CEST describes the situation where magnetization is transferred between water protons residing in different compartments. Early CEST studies demonstrated this behavior by applying a frequency-selective pulse that presaturates the solute proton pool. The saturation is then transferred to the bulk water via chemical exchange, resulting in a decrease in water signal\textsuperscript{11} (Figure 6.2).

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{cest_diagram.png}
\caption{Schematic representations of the distribution of spins, aligned with and against the field (upper and lower energy levels, respectively) \textit{(above)} and simulated NMR spectra \textit{(below)} for two chemically distinct pools of nuclei (left), two spins after a saturation pulse has been applied to one pool (middle), and for a system undergoing chemical exchange after a saturation pulse has been applied to one pool (right). Figure and caption reproduced from Sherry and Woods\textsuperscript{11}.}
\end{figure}

pH affects the chemical exchange process by altering the rates at which protons between different compartments are exchanged. The rates of chemical exchange are catalyzed by the presence of both H\textsuperscript{+} and OH\textsuperscript{-} ions coordinated to the polymer\textsuperscript{12,13}, meaning that the chemical exchange rate is proportionally faster at
pH values differing from the molecule’s pK\textsubscript{a}/pK\textsubscript{b}. Since chemical exchange is catalyzed by both acid and base, a plot of the chemical exchange rate against pH would show a “V-shaped” curve. The non-monotonic behavior is typical of many chemical exchange processes and necessitates a careful selection of the molecule for sensing purposes. A material with overlapping exchange rates in the pH range of interest could lead to confounding effects and make it difficult for accurate pH determination.

Spin exchange between macromolecules and interfacial water protons alters the measured relaxation time of water molecules. While macromolecule protons are normally “invisible” in the MRI due to their short transverse relaxation times, a transfer of their magnetization to water molecules, either through direct proton exchange or dipole-dipole interactions, can shorten the relaxation rate of water molecules\textsuperscript{14}. The extent of this relaxation rate modification depends on the type of surface functional groups on the macromolecules. pH-responsive functional groups, such as carboxyl or hydroxyl moieties, that contain exchangeable protons with a pH-dependent exchange rate can be used to alter the relaxation rate of absorbed water protons in a pH-dependent manner. The varying exchange rates at different pH translates into a pH-dependent relaxation rate alteration in these material systems\textsuperscript{15}. This chapter describes a method incorporating a similar concept in hydrogels with a pH-dependent transverse relaxation rate that can be measured using a regular CPMG pulse sequence\textsuperscript{15} (Figure 6.3).
Figure 6.3: The transverse relaxation time of hydrogels and biomolecules with specific functional groups change with pH. These materials are used as a starting point for developing our pH sensors. Reference: Gochberg, et al., *J Magn Reson* 131, 191-198 (1998).

6.4 PolyHEMA as a pH-sensitive material

Hydroxyethylmethacrylate (HEMA) is a material best known for its use in contact lenses. Its proven biocompatibility makes it an attractive candidate as an implantable material. Poly-Hydroxyethylmethacrylate (HEMA) hydrogels were synthesized by crosslinking Hydroxyethylmethacrylate with N,N'-Methylenebisacrylamide (BIS). The reaction was catalyzed by the addition of free radical initiators in an oxygen-free environment. The synthesized hydrogel was immersed in DI water for 24 hours to wash out any unreacted monomer units. Sensor response was measured as changes in transverse relaxation time ($T_2$) with pH in the Bruker Minispec. Gel pieces were left soaking in pH buffers overnight at 40°C to ensure that equilibrium was reached.
The effect of HEMA concentration in the reaction mixture was examined in order to determine the optimal sensor composition. The amount of HEMA was modulated while the amount of BIS was kept constant. This resulted in a change in the concentration of HEMA surface groups that water has access to. Increasing the concentration of HEMA monomer in the reaction mixture increases the pH sensitivity of the hydrogels, as reflected in a positive correlation between monomer concentration and the extent of change in relaxation rate with pH (Figure 6.4.1). Additionally, the measured relaxation rates are correlated to the concentration of HEMA in the hydrogel (Figure 6.4); the gel made with 24% HEMA has the fastest relaxation rate at all pH values. This effect is likely due to a combination of increased chemical exchange, increased coordination of water molecules on the polymer chain, and a different physical structure of the hydrogel.

Figure 6.4: The transverse relaxation rate increase with increasing concentration of HEMA, and the response to changing pH values also increases with the concentration of HEMA in the reaction mixture. These effects are due to increased concentration of active CEST sites on the polymer and changes in the physical structure of the polymer.
The relaxation rate can be observed to decrease monotonically between pH 5 and pH 6.5. Between pH 6.5 and 7.4, the relaxation rate increases again. This V-shaped behavior has several likely explanations. First, a secondary exchange site could have become active at higher pH values. Each polymer molecule contains a number of exchangeable protons, and it’s possible that some of them would became active at higher pK_a compared to others\textsuperscript{15}. A second possible explanation involves base-catalyzed chemical exchange at pH values higher than the pKa of HEMA. The catalyzed exchanged process results in decreased relaxation time at those pH values.

The effect of crosslinker concentration in the reaction mixture was examined by decreasing the concentration of BIS from 3% to 1%. This change resulted in a higher T_2 at all pH levels (Figure 6.5), irrespective of HEMA concentrations. A change in crosslinking ratio primarily affects the architecture of the hydrogel and the same behavior in relaxation rate has previously been observed in other polymer systems\textsuperscript{14}.

The intended application of this technology is detection of response to therapy in tumors, where a detection range of pH5 – pH6.5 is appropriate. The pH response behavior is governed by the pK_a of side groups located on the polymer chains, as shown in the previous section, and the selection of a different monomer / crosslinker combination could be an effective method to modify the pH response to cover a different range.
Figure 6.5: The transverse relaxation times of HEMA-BIS hydrogels are determined by both the concentration of HEMA and concentration of BIS in the reaction mixture. The relaxation times observed in the more highly crosslinked polymer (top) are lower at all pH values compared to similar polymers (bottom) with the same HEMA content. The overall shape of pH response, however, remained similar at different crosslinking levels.
The response dynamics of these gels were studied on the AutoNMR relaxometer. Gel pieces were encapsulated in enclosures made of polyethylene and secured with a size-exclusion membrane. The polyethylene shells of the device contained the hydrogel while the membrane facilitated transport of solutes into the gel (Figure 6.6). Sensor response and reversibility were recorded as the buffer was switched from pH 7.4 to pH 5.5 and then back.

Figure 6.6: The polyethylene shell enclosing the HEMA-BIS gel ensures that proton transport into the gel can only occur from the opening at the top. These devices were used to evaluate the kinetics of change of the pH gels.

Figure 6.7 shows the time course response of 3,6, and 12% HEMA, 1% BIS gels. Steady state was achieved in two hours and the response was completely reversible. This response time suggests a diffusion rate of roughly $3 \times 10^{-6} \frac{cm^2}{s}$ for hydrogen ions in the gel, which is an order of magnitude faster than reported hydrogen diffusion rates in other hydrogels (generally around $10^{-8} \frac{cm^2}{s}$). This fast diffusion can be explained by the physical structure of the synthesized gel. Figure 6.8 contains a representative electron micrograph of a 12% HEMA 1% BIS gel. The hydrogel is comprised of beads of polymeric material separated by tunnels measuring microns in size. The larger effective surface area from these interconnected pores results in fast diffusion compared to non-macroporous hydrogels. This macroporous structure has its origins in the synthesis condition. It has previously been shown that polymerization of HEMA in 60% - 90% water results in a macroporous structure. This phenomenon is driven by the decreasing
water solubility of polymerized HEMA subunits; as polymer chains grow longer, phase separation takes place and polymer aggregates precipitate out of solution. The end result is a “sponge” consisting of aggregated microspheres.\(^\text{18}\).

Figure 6.7: The measured transverse relaxation time of the pH gels equilibrate within 2 hours of a step change in pH. The pH was alternated between 7.4 and 5.5 in this experiment, and the results were measured on the NMR MOUSE setup. Each data point represented the averaged result of 3 devices.
6.5 pH measurements using micro-resonator device

The micro-resonator device (Figure 6.9) is a coupled wireless NMR transceiver designed for single voxel sensing\(^\text{19}\). This sensor device has a hollow center where different types of contrast-generating medium, such as the pH-responsive hydrogel mentioned above, reside. The contrast agents are excited with the use of an external, single sided magnet and measured with a coupled reader. The setup can measure both the longitudinal and transverse relaxation rates of the contrast material contained within the microsreonator and effectively performs single voxel imaging\(^\text{19}\). The sensors were filled with 24% HEMA, 1% BIS hydrogel prepolymer that was polymerized using the same redox reaction (described above) under nitrogen at room temperature.
Figure 6.9: The microresonator device measures roughly 1.5mm in diameter and can be injected through the bore of a biopsy needle\textsuperscript{19}. The sensors are coupled to an external reader that allows for MR excitation and measurements.

The wireless sensor devices were characterized with pH-adjusted saline using a recirculating water bath. The data was acquired over approximately 25 days and can be seen in Figure 6.10. The sensor exhibited excellent stability over the 25-day period and thus is suitable for long-term measurement tasks. The change in $T_2$ with pH can be modeled as a weak acid\textsuperscript{19} (Figure 6.11) and fitted to the equation:

$$\frac{1}{T_2} = \frac{1}{T_{2,0}} + \frac{1}{T_{2,b}(1 + 10^{pH-pK_a})}$$

where $T_{2,0}$ and $T_{2,b}$ represent constants that are unique to the polymer system. The system has shown a pH sensitivity of 0.1 pH unit\textsuperscript{19}.
Figure 6.10: The HEMA-BIS gel detected changes in pH in a microresonator device for 3 weeks. The devices was placed in a tube where pH-adjusted saline from a temperature controlled bath flows through. The bath temperature and pH were monitored using an external pH probe.
Figure 6.11: The response of HEMA-BIS gel can be modeled similarly to a weak acid.

6.6 Tumor Model

A pilot study was performed in C57/BL6 mice with the wireless sensing device. This study was designed to validate sensor performance after implantation in the tumor and estimate the effect size generated by the tumors. Figure 6.12 shows the result from this study. The device implanted in the tumor was found to have a lower T\textsubscript{2} compared to devices implanted next to the tumor or implanted in the flank with no tumor. The lower T\textsubscript{2}, corresponding to a lower pH value, correlates to the expectation that tumor tissue is more acidic than normal tissue.
Figure 6.12: Devices implanted inside the tumor showed a lower transverse relaxation time compared to devices implanted near the tumor or at the contralateral flank.

We next tested these devices in the tumor model described earlier in this chapter. A group size of 6 was chosen based on results from a pilot study\textsuperscript{19}. After tumors were implanted in these mice, they were divided into an experimental group where they received daily intraperitoneal injections of Doxorubicin and a control group where they received daily intraperitoneal injections of saline. The dosage of drug was set at 2mg/kg to avoid toxic side effects. We performed sensor measurements daily for up to 4 days after sensor implantation; the extracted $T_2$ values were found not to be significantly different the experimental group and the control group.

A separate tumor induction study provided answers for these confounding results. The dosage of Doxorubicin administered to animals was increased to 10mg/kg in this study. Figure 6.13 shows two sets of Hexamtoxylin and Eosin (H&E) stained...
slices of tumors taken from mice dosed with saline and 10 mg/kg Doxorubicin daily, respectively. The overall observation is that significant heterogeneity exists within each tumor; particularly, large patches of necrotic areas (empty looking tissue structure without cell nuclei) can be seen throughout the slide taken from both sets of animals. In the tumor dosed with 10mg/kg, areas of apoptosis (cells with condensed nuclei), indicative of chemotherapeutic effect, are visible in the tumor, but the areas affected by apoptosis are small compared to the size of necrotic areas.

Figure 6.13: H&E stained histographs of animals that received Doxorubicin (right) are virtually indistinguishable from animals that received saline (left) injections. The extensive necrotic regions (blanks and light pink areas) seen in both sets of pictures produced the confounding results described.

Intratumoural, extracellular pH can be regarded as a general indicator of cell death in the tumor and thus cannot be used to distinguish between cell death caused by necrosis and apoptosis. Tumors with extensive necrotic areas are poor models for
this mode of sensing and can lead to confounding results such as that described in this section (Figure 6.14, Figure 6.15). The primary driver behind tumor necrosis is disorganized vasculature in the tumor. The lack of normal tissue architecture results in parts of the tumor becoming necrotic over time due to insufficient oxygen and nutrient delivery. Necrosis tend to be a more severe problem in rapidly proliferating tumors or tumors that have grown to a large size, mainly because new vasculature formation cannot keep up with the rapid pace of tumor growth or cannot penetrate to the core of a large solid tumor.

Figure 6.14: Measured transverse relaxation time of sensors implanted in mouse tumors. Red lines denote animals that received daily Doxorubicin IP injections and black lines denote animals that received daily saline IP injections.
Figure 6.15: The difference between relaxation time measured on the first day (pre-dosing) and on the day of animal sacrifice. The results did not show statistical significance between animals dosed with drug and animals dosed with saline.

A fundamental challenge in this animal model, therefore, is the need to minimize the amount of necrosis in the tumor. Selection of a resistant cell line with slower proliferation rate or better vascularization should help reduce the amount of necrosis found in control tumors and minimize this confounding effect. A sensitive cell line with a more robust response to the drug of interest would also help by increasing the effect of treatment.
6.7 Conclusion

The ability to detect pH changes in the range of pH 5 – pH 6.5 was demonstrated with pH sensors based on HEMA-BIS hydrogels. The chemical exchange rate between the surface functional groups found on these gels and water is pH dependent and results in a changing transverse relaxation rate that can be measured with MR techniques. The HEMA-BIS gels were tested both \textit{in vitro} and \textit{in vivo} as part of the microresonator sensor system. The sensor detected pH changes in saline for 3 weeks in a flow cell, and detected the acidified pH in the center of a tumor. The pH sensors are designed to detect pH change due to general cell death, and a cancer model with small necrotic areas is needed for the sensor to be tested in.
References