FLUORINE-19 (\(^{19}\text{F}\)) MAGNETIC RESONANCE IMAGING OF PULMONARY VENTILATION, CELL MIGRATION, AND INFLAMMATION

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ABSTRACT

Esther Olabosede Akinnagbe-Zusterzeel: Fluorine-19 (\textsuperscript{19}F) magnetic resonance imaging of pulmonary ventilation, cell migration and inflammation
(Under the direction of Yueh Z. Lee)

Conventional \textsuperscript{1}H magnetic resonance imaging (MRI) has long been used in a variety of clinical applications. Among other advantages, MRI lacks ionizing radiation while still providing high resolution images with significant soft-tissue contrast. Advances in coil design and pulse sequencing have allowed for the adaptation of clinical scanners to fluorine-19 based MRI (\textsuperscript{19}F MRI). Well-understood to be inert and non-toxic, many commercially available \textsuperscript{19}F compounds can be introduced into subjects as tracing agents. Resulting images have high signal-to-noise ratios (SNR) due to minimal intrinsic \textsuperscript{19}F signal; any signal recorded originated from the introduced agent. \textsuperscript{19}F MRI presents several new opportunities to improve and create applications of an already versatile imaging modality.

**Pulmonary Imaging** As the need to understand the physiology of abnormal pulmonary conditions grows, so does the demand for quantitative lung function imaging methods. The use of exogenous \textsuperscript{19}F gas along with improved pulse sequencing and gradient hardware has improved the once low-quality lung MR images caused by low water molecule density in lung tissue. This study aimed to demonstrate pulmonary ventilation imaging in healthy controls and cystic fibrosis (CF) patients using \textsuperscript{19}F MRI to study ventilation kinetics. \textsuperscript{19}F MR images of twelve adult
subjects performing respiratory exercises were acquired using a Siemens TIM Trio 3 Tesla scanner following spirometry. Ventilation kinetics within the lung space were computed by fitting dynamic $^{19}$F signal intensity values to a six-parameter fit model. Statistical analysis of ventilation kinetic between groups revealed that CF lungs required significantly more time to fill and showed ventilation defects. $^{19}$F imaging presents an inexpensive method of quantitatively measuring lung function in both healthy and affected populations, creating the opportunity to test treatment efficacy of various pulmonary disease as well as structural and physiological lung measurements.

**Cell Tracking** One emerging application of $^{19}$F MRI is cell tracking using perfluorocarbon probes (PFC). Human and murine cells were labeled with the PFC probe to explore feasibility of $^{19}$F MRI cell tracking and inflammatory response imaging in a CF cell model and murine Parkinson’s disease (PD) model. Studies validated the uptake of the labeling agent by all cell types, showing minimal decrease in cell survival rates. $^{19}$F NMR and MRI techniques were employed to analyze loading efficiency and for cell quantification *in vivo*.

We demonstrate preliminary imaging data of fluorine-19 gas and compounds suitable for pulmonary imaging and cell tracking. High signal to noise ratios are achievable with small animal imaging MR scanners as well as conventional 3T human systems with high bandwidth amplifiers.
# TABLE OF CONTENTS

List of Tables ......................................................................................................................... viii

List of Figures ........................................................................................................................ ix

List of Abbreviations ............................................................................................................. x

Chapter One: Introduction .................................................................................................... 1

Magnetic Resonance Imaging Principles .............................................................................. 1

Formation of MRI Signal ...................................................................................................... 2

MRI Signal Detection .......................................................................................................... 4

Spin-Lattice and Spin-Spin Relaxation ................................................................................... 5

Magnetic Field Gradients, Spatial Localization, and Image Formation ................................. 7

References ............................................................................................................................ 9

Chapter Two: Fluorine-19 Magnetic Resonance Imaging of Pulmonary Ventilation in Cystic Fibrosis Patients .................................................................................................. 10

Introduction .......................................................................................................................... 10

Methods ................................................................................................................................. 12

Spirometry ............................................................................................................................. 12
Chapter Three: Fluorine-19 Magnetic Resonance Imaging for Cell Tracking and Inflammation Imaging ................................................................. 27
  Introduction ........................................................................................................ 27
  Methods .................................................................................................................. 29
    Cystic Fibrosis Model Cell Culture Procedures ...................................................... 29
    Parkinson’s Disease Model Cell Culture Procedures ............................................ 30
  Animals .................................................................................................................... 30
  Cytotoxicity and Fluorescence Measurements ....................................................... 31
  NMR Spectroscopy .................................................................................................. 32
  MR and Fluorescence Imaging .................................................................................. 33
  Results ...................................................................................................................... 34
    Cystic Fibrosis Model Results .............................................................................. 34
LIST OF TABLES

Table 1. 1. NMR properties of select isotopes .................................................. 2

Table 2. 1. Subject Characteristics....................................................................... 16

Table 3. 1 Pairwise t-test p-values of fluorescence for THP-1 and HBE cells .......... 35

Table 3. 2. PFC probe loading efficiency ................................................................. 36

Table 3. 3. In vivo murine BMM quantification over time ...................................... 40
LIST OF FIGURES

Figure 1. 1. Origin of NMR signals ................................................................. 3
Figure 1. 2. Stationary versus rotating reference frame ...................................... 5
Figure 2. 1. Proton MRI of human thorax. ............................................................. 17
Figure 2. 2. Coronal 19F MR images of a representative healthy control .................. 18
Figure 2. 3. 1H and 19F MRI fusion image ............................................................ 18
Figure 2. 4. Time to steady-state color maps .......................................................... 19
Figure 2. 5. The distribution of voxel TSS ............................................................. 19
Figure 3. 1. Fluorescence images of labeled THP-1 cells and CRC-grown HBEs .... 33
Figure 3. 2. Mean plate reader fluorescence measurements for THP-1 and HBE cells .. 35
Figure 3. 3. WST-1 assay toxicity measurements for THP-1 and HBE cells .............. 36
Figure 3. 4. 19F NMR spectra for pelleted and labeled cells .................................. 37
Figure 3. 5. 19F MRI of cell-pellet phantoms and reference phantom ...................... 38
Figure 3. 6. Toxicity measurements for burine BMM ............................................. 39
Figure 3. 7. Axial proton MRI, 19F MRI, and their fusions of murine BMM ............. 40
# LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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</thead>
<tbody>
<tr>
<td>$^1$H</td>
<td>Hydrogen-1</td>
</tr>
<tr>
<td>$^3$He</td>
<td>Helium-3</td>
</tr>
<tr>
<td>$^{19}$F</td>
<td>Fluorine-19</td>
</tr>
<tr>
<td>$^{129}$X</td>
<td>Xenon-129</td>
</tr>
<tr>
<td>AD</td>
<td>Alzheimer’s disease</td>
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<tr>
<td>BBB</td>
<td>Blood-brain barrier</td>
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<tr>
<td>BMM</td>
<td>Bone marrow-derived macrophage</td>
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<tr>
<td>CF</td>
<td>Cystic fibrosis</td>
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<tr>
<td>CFTR</td>
<td>Cystic fibrosis transmembrane conductance regulator</td>
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<tr>
<td>CNS</td>
<td>Central nervous system</td>
</tr>
<tr>
<td>COPD</td>
<td>Chronic obstructive pulmonary disease</td>
</tr>
<tr>
<td>CRC</td>
<td>Conditionally-reprogrammed cell</td>
</tr>
<tr>
<td>CT</td>
<td>Computed tomography</td>
</tr>
<tr>
<td>FEV$_1$</td>
<td>Forced expiratory volume in one second</td>
</tr>
<tr>
<td>FOV</td>
<td>Field-of-view</td>
</tr>
<tr>
<td>GDNF</td>
<td>Glial cell-derived neuronal growth factor</td>
</tr>
<tr>
<td>HBE</td>
<td>Human bronchial epithelial</td>
</tr>
<tr>
<td>HP</td>
<td>Hyperpolarized</td>
</tr>
<tr>
<td>HRCT</td>
<td>High-resolution CT</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic Resonance Imaging</td>
</tr>
<tr>
<td>OE</td>
<td>Oxygen-enhanced</td>
</tr>
<tr>
<td>PD</td>
<td>Parkinson’s disease</td>
</tr>
<tr>
<td>Acronym</td>
<td>Description</td>
</tr>
<tr>
<td>---------</td>
<td>----------------------------------</td>
</tr>
<tr>
<td>PFC</td>
<td>Perfluorocarbon</td>
</tr>
<tr>
<td>PFP</td>
<td>Perfluoropropane</td>
</tr>
<tr>
<td>PFPE</td>
<td>Perfluoropolyether</td>
</tr>
<tr>
<td>PFT</td>
<td>Pulmonary function test</td>
</tr>
<tr>
<td>RF</td>
<td>Radio frequency</td>
</tr>
<tr>
<td>SNR</td>
<td>Signal-to-noise ratio</td>
</tr>
<tr>
<td>$T_2^*$</td>
<td>Effective transverse relaxation time</td>
</tr>
<tr>
<td>TE</td>
<td>Echo time</td>
</tr>
<tr>
<td>TSS</td>
<td>Time to Steady-State</td>
</tr>
<tr>
<td>VDV</td>
<td>Ventilation defect volume</td>
</tr>
<tr>
<td>V/Q</td>
<td>Ventilation-perfusion</td>
</tr>
<tr>
<td>$\alpha$</td>
<td>Flip angle</td>
</tr>
<tr>
<td>$\omega$</td>
<td>Larmor frequency</td>
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</table>
CHAPTER ONE: INTRODUCTION

Magnetic Resonance Imaging Principles

The basic physical principles of nuclear magnetic resonance (NMR) form the foundation of magnetic resonance imaging (MRI). Purcell *et al.* [1] and Bloch *et al.* [2] independently demonstrated the application of those principles to MRI in 1946 [1, 2]. Paul C. Lauterbur and Peter Mansfield shared the 2003 Nobel Prize in Physiology or Medicine due to their research in the 1970s relating to MRI. Lauterbur discovered that additional magnetic field gradients could enable creation of crude two-dimensional (2D) images. He was able to demonstrate that the extra gradients allowed him to create a cross-sectional image of water filled tubes, and that he was able to distinguish between the two types of water (ordinary and heavy) in each tube. Mansfield was able to better demonstrate resonance difference using gradients, and he discovered how to quickly analyze and transform signals into images. The field of MRI technology has developed into a diverse and widely used tool for research and clinical applications.

To facilitate discussion of MRI acquisition techniques for pulmonary imaging and cell tracking in this dissertation, the following will provide a brief review of the basic physical principles of NMR. Readers can refer to “*Magnetic Resonance Imaging: Physical Principles and Pulse Sequence Design*” by Haacke *et al.* [3] for further details on NMR and MRI physics and image construction.
**Formation of MRI Signal**

The NMR signal originates from a property of many subatomic particles known as “spin”. Only nuclei with an odd number of protons or neutrons exhibit spin angular momentum, \( \vec{J} \).

\[
\vec{J} = \hbar \vec{I} \quad [1-1]
\]

\( \vec{J} \) is represented as the product of the reduced Planck's constant, \( \hbar \) and the intrinsic half-integer spin, \( \vec{I} \). The spinning of the nucleus, which has both charge and mass causes the nucleus to behave like a spinning charge, giving rise to a magnetic dipole moment, given by:

\[
\vec{\mu} = \gamma \vec{J} = \gamma \hbar \vec{I} \quad [1-2]
\]

where \( \gamma \) represents the unique gyromagnetic ratio for each nucleus; gyromagnetic ratios for some NMR-sensitive nuclei are listed in Table 1. 1.

<table>
<thead>
<tr>
<th>Nucleus</th>
<th>Spin</th>
<th>Gyromagnetic ratio (MHz/T)</th>
</tr>
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<tbody>
<tr>
<td>Hydrogen (proton), (^1)H</td>
<td>1/2</td>
<td>42.577</td>
</tr>
<tr>
<td>Helium-3, (^3)H</td>
<td>1/2</td>
<td>-32.44</td>
</tr>
<tr>
<td>Oxygen-18, (^17)O</td>
<td>5/2</td>
<td>-5.77</td>
</tr>
<tr>
<td>Fluorine-19, (^19)F</td>
<td>1/2</td>
<td>40.08</td>
</tr>
<tr>
<td>Xenon-129, (^{129})Xe</td>
<td>1/2</td>
<td>-11.86</td>
</tr>
</tbody>
</table>

Spins placed in an external magnetic field (\( B_0 \)) tend to align with that field, thus creating a net magnetization density that can be treated as a vector sum of the magnetic dipole moments (Figure 1. 1c) in a volume (\( M_0 \)). Figure 1. 1d depicts a diagram of the bulk magnetization vector driven toward the positive z-direction. In this schematic, a single proton is described as a spinning sphere with a mass and charge as well as an intrinsic spin, shown as a rotation of the nucleus about an axis (Figure 1. 1a). The combination of the spin and charge causes the nucleus to exhibit magnetic properties not unlike a magnetic bar aligned with \( B_0 \) (i.e. a dipole moment).
The angular momentum caused by the spin “tips” the dipole moment way from its vertical alignment, causing it to precess or “wobble” about the vertical axis (Figure 1. 1b) at the precessional frequency known as the Larmor frequency ($\omega$).

The bulk magnetization vector can also be described by the differences in energy level within a population of dipole moments. The potential energy of a single nucleus, $P_E$ can be expressed as a function of the angular moment for said nucleus and $B_0$, or more specifically as a function of intrinsic spin, gyromagnetic ratio, and $B_0$. Half spin nuclei, such as $^1$H or $^{19}$F, can either spin parallel or anti-parallel to the external magnetic field. In other words, each dipole moment can have a spin value of $I = \pm1/2$. Spins aligned parallel to the magnetic field occupy a lower energy state, causing there to be slightly more spins aligned parallel to the magnetic field. Without the exposure to an external magnetic field, half-spin nuclei return to an even distribution.
of parallel and anti-parallel alignment, eliminating the net polarization or magnetic effect. At a clinical magnetic field strength of 3.0 T, the net polarization of hydrogen atoms at body temperature is about $9.9 \times 10^{-6}$—this polarization though small still provides a detectible signal due to the high concentration of $^1$H nuclei in human and animal tissue. The polarization yielded for fluorine-19 ($^{19}$F) nuclei under the same conditions is nearly the same ($9.3 \times 10^{-6}$), however the concentration of fluorine atoms in human tissue is 4 µM versus 88 M for $^1$H nuclei. Therefore, it is essential to utilize exogenous fluorinated contrast agents to acquire a signal for $^{19}$F MRI.

**MRI Signal Detection**

Precessing magnetization is detected through a pair of receiver coils tuned to the larmor frequency, which are typically aligned to the transverse (x-y) plane. The transverse plane is defined as the plane perpendicular to the applied external field. The transverse component of the time varying magnetic field is coupled with the receiver coils, inducing a signal that is proportional to the rate of change of the magnetization detected at the coil. Because the two coils are positioned orthogonal to the x- and y-components of the transverse plane, the direction of rotation and angle of transverse magnetization are measurable over time.

At equilibrium, net magnetization ($M_0$) is parallel to the $B_0$ field (along the z-axis), meaning there is no transverse plane magnetization component generating a signal. NMR signals are generated by the application of a radio frequency (RF) pulse that applies a time-varying magnetic field, $B_1$ orthogonal to the main magnetic field, $B_0$. In other words, an RF pulse can be represented as a magnetic field vector of a set magnitude that rotates about the z-axis on the x-y plane at the Larmor or excitation frequency. It is essential that the $B_1$ field rotates at the larmor frequency in order for the nuclei to be excited. The nature of the rotating magnetic
field, $B_1$ allows the ensemble of spins in the sample to progressively absorb energy and nutate away from the longitudinal axis ($z$-axis), creating the time-varying transverse component. The angle of nutation or flip angle ($\alpha$) depends on the magnitude of the RF pulse as well as the duration of its application to the sample. Due to the complex nature of the bulk magnetization vector motion, the spin system can be depicted as in Figure 1. 2, with a special time-varying reference frame. In this system, the frame of reference itself rotates about the $z$-axis at the Larmor frequency, making the depiction of complicated RF pulse excitation-relaxation motion possible. After the RF pulse is applied, receiver coils measure the decay of the transverse magnetization component and the recovery of the longitudinal magnetization component.

![Diagram showing rotating magnetization field](image)

*Figure 1. 2. The rotating magnetization field induces an oscillating signal at frequency $\omega$ as viewed in the (a) stationary frame and (b) rotating reference frame.*

**Spin-Lattice and Spin-Spin Relaxation**

Spin-lattice relaxation describes the process by which the net magnetization returns to thermal equilibrium along the longitudinal axis. This relaxation mechanism occurs when an individual nucleus attempts to reach a lower energy state (parallel alignment with $B_0$ field) following an RF pulse. Transitions from higher to lower energy states result in dissipation of energy (in the form of thermal motion) into the lattice of nearby atoms. The thermal motion of
atoms creates time-dependent magnetic fields with frequencies close to \( \omega \). This in turn allows energy state transition, which relaxes the sample back to thermal equilibrium. Spin-lattice relaxation is characterized by the \( T_1 \) relaxation time constant; it is defined as the time required to reach 63% of the equilibrium longitudinal magnetization.

The transverse magnetization component is made up of the sum of the individual vectors from individual spins in the x-y plane during \( B_1 \) field application. Directly following the RF pulse, these individual spins begin their dephasing process, known as spin-spin relaxation. This mechanism is caused by unavoidable changes in magnetic field spatial distribution due to the introduction of the tissues themselves into the magnetic field. Interactions between proximal spins cause them to “speed up or down” due to their inherent magnetic property—magnetic susceptibility. The difference in susceptibility of varying materials in a sample lead to subtle inhomogeneities in the field, which leads to dephasing. In other words, the slightly heterogeneous magnetic field causes tissues at different locations to precess at slightly different Larmor frequencies. At high fields, susceptibility artifacts are exaggerated because variations in magnetic susceptibility is proportional to the magnetic field strength. The end result is that the net magnetization eventually decreases.

The transverse relaxation time constant, \( T_2 \) characterizes spin-spin relaxation; it is caused by the inherent transverse dephasing due to the presence of nearby spins. \( T_2^* \) incorporates perturbations in the \( B_0 \) field which further decreases the transverse magnetization as well as the heterogeneous magnetic field caused by nearby spins; in other words, it takes into account both static and time-dependent effects. \( T_2^* \) is typically much shorter than \( T_2 \). Unlike \( T_1, T_2 \) time-varying changes occur at a much lower frequency range and are effected by variations in all three directions.
T₁, T₂, and T₂* relaxation time constants are properties that are intrinsic to material. It is the very difference in these properties that creates the contrast between material and tissues in MR images.

*Magnetic Field Gradients, Spatial Localization, and Image Formation*

Unlike other medical imaging modalities, the energy measured in MRI does not contain any intrinsic location information. It is therefore necessary to employ other techniques (multiple gradients, Fourier transform, etc.) to provide geographical information. In order to spatially localize received signals, known linear magnetic field gradients are applied in conjunction with the B₀ field. These gradients are applied in the x-, y-, and z-directions and can vary in application time, position, and gradient strength to create intentional magnetic field inhomogeneities.

Slice selection is performed using a linear and non-RF magnetic field gradient applied in the z-direction during the RF excitation. The Larmor frequency then becomes a function of location on the z-direction magnetic gradient (Gₜ). Applying a band-limited RF excitation ensures that the received signal originates from a particular slab (or slice). Frequency encoding employs a process similar to slice selection; a magnetic gradient is applied in a direction transverse to the z-direction. This gradient is applied in such a way that its range fits into the bandwidth of that particular slice so that when readout occurs, there is additional spatial information within the slice. Phase encoding occurs in the remaining direction (typically the shortest dimension) and usually occurs neither during RE excitation nor reception. For phase encoding, the idea is not to change the frequency of the signals, but to allow for dephasing to occur linearly.
Because MRI signals detected by receiver coils are time-varying oscillations, they are best described as sinusoidal functions with unique amplitude, frequency, and phase. Spatially encoded native MRI signals are obtained in the frequency domain, necessitating the use of inverse Fourier transforms to create an image in the spatial domain. The k-space representation of an image depicts an object in the frequency domain; it is “filled” with the signal from receiver coils.

The filling of the k-space can vary by application, and the process is defined by the image sequence used. Image pulse sequences in MRI refer to the pattern of application of RF pulses and gradients. It is the variation in the RF pulse and gradient strength, frequency and duration that determine the location in space of an excitation, and therefore the resulting signal. As such, the time-varying magnetic signal recorded is spatially encoded through time. In other words, the k-space is a graphic representation of the area under the x- and y-gradients that are proportional to the spatial frequencies of those axes. Inverse fourier transformations of the filled k-space allow the conversion of these special frequencies to spatial location.

While this provides a brief overview of the principles of conventional MR imaging, these principles also apply to multinuclear MRI. In $^{19}$F MRI, exogenous fluorinated contrast agents are introduced to biological tissue. Coils specially tuned to the $^{19}$F isotope are a relatively inexpensive and simple to construct, allowing current clinical scanners to be used in $^{19}$F MRI applications. In essence, implementation of $^{19}$F MRI techniques is relatively easy to achieve through improved image pulse sequence and dual-tuned coil hardware.
REFERENCES


CHAPTER TWO: FLUORINE-19 MAGNETIC RESONANCE IMAGING OF PULMONARY VENTILATION IN CYSTIC FIBROSIS PATIENTS

Introduction

Cystic Fibrosis (CF), is an autosomal recessive genetic disease affecting multiple tubular organ systems, which commonly causes persistent pulmonary infections and severely decreases lung function [1]. Pulmonary failure, in conjunction with complications in the liver, pancreas and intestines characterize this disease—the most common life-limiting heritable disease amongst Caucasians [2, 3]. Caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene [1-2, 4], patients with CF inherit two sets of mutated CFTR genes—one from each parent [1, 2]. The genetic defects of CFTR genes mainly prevent proper transport of chloride and bicarbonate ions. It is believed that the interaction between defective CFTR ion channels and epithelial sodium channels causes airway dehydration, disrupting mucociliary clearance [4]. This provides an ideal environment for bacterial growth in the lungs, prevents nutrient uptake due to pancreatic damage, and leads to a host of problems caused by electrolyte imbalances in the patient [1-3]. The chronic infection and inflammation of the pulmonary system contribute to the obstruction of airways, leading to bronchiectasis and irreversible lung damage [1]. Measuring and recording inflammatory damage to airways caused by defective mucus clearance is of prime importance in CF research.

There is an increasing need in CF research to develop methods of acquiring physiological information as well as anatomical data of the lungs for treatment efficacy assessment and early lung disease detection. Current gold standards of functional pulmonary assessment include pulmonary function tests (PFTs), lung computed tomography (CT) for anatomical image
acquisition and scintigraphy to obtain measurements of pulmonary function, i.e. ventilation-perfusion (V/Q) scans [5-8]. Clinically, spirometry measurements (forced expiratory volume in one second; FEV\textsubscript{1})—a type of PFT—is widely used to predict outcomes in moderate to severe CF patients [5, 9, 10]. Spirometry measurements are limited however, by their lack of local functional information, which is crucial in hetereogenous diseases such as cystic fibrosis. Conventional pulmonary imaging techniques have the advantage of providing morphological and some functional data [6-8], but are limited by patient exposure to ionizing radiation.

Unlike the current methods of pulmonary imaging, MRI-based methods are not limited to static global measurements, thus allowing the investigation of regional physiological and pathological changes over a period of time [12, 13]. Furthermore, MRI-based techniques present a noninvasive method of quantitatively measuring pulmonary function without exposing subjects to ionizing radiation while increasing image acquisition speed. Because traditional MRI techniques detect their signal from hydrogen-1 (\textsuperscript{1}H) protons, low water-molecule density and high susceptibility in the lungs results in low-quality pulmonary images. With the advancement of multi-nuclear hardware and better pulse sequences, the use of exogenous gases as a contrast agent has greatly improved the suitability of MRI techniques for pulmonary imaging.

Hyperpolarized gases, such as helium-3 (\textsuperscript{3}He) and xenon-129 (\textsuperscript{129}Xe) are promising choices for an exogenous gas, mainly because of their relatively high signal-to-noise ratio (SNR) [11]. Fluorinated gases— unlike hyperpolarized gases— do not require a polarizing device, thus reducing costs and can be mixed with oxygen [14]. Compounds containing fluorine-19 (\textsuperscript{19}F) are particularly attractive as a contrast agent not only because they are inert and non-toxic, but also because the infinitesimal intrinsic \textsuperscript{19}F signal occurring biologically does not hinder ventilation.
measurements [15]. This study focuses on the use of a $^{19}$F exogenous gas, perfluoropropane (C$_3$F$_8$; PFP) to enhance pulmonary image acquisition through $^{19}$F magnetic resonance imaging ($^{19}$F MRI). PFP is an ideal $^{19}$F contrast agent because of low absorption rate by tissue due to low water solubility, and its relatively low spin-lattice relaxation time ($T_1$; 12.4 ms), which aids rapid image acquisition [16].

Previous studies have demonstrated the safety and efficacy of PFP (79%) with oxygen (21%) as a contrast agent in $^{19}$F MRI in healthy subjects, chronic obstructive pulmonary disease (COPD) patients, asthma patients and lung transplant recipients [15, 16]. In this study, the regional ventilation kinetics and ventilation defect identification is explored in healthy subjects and those with cystic fibrosis. The goal of this study was to gather regional pulmonary ventilation information by means of multiple-breath $^{19}$F MRI acquisitions with sufficient $^{19}$F signal strength and image contrast.

**Methods**

The University of North Carolina Institutional Review Board approved this study. Following informed consent by the subjects, 6 healthy volunteers and 8 cystic fibrosis patients (FEV$_1$>30%) received a clinical assessment and spirometry. $^{19}$F MRI scans were then performed to obtain images of the gas wash-in and wash-out.

**Spirometry**

A trained technician performed spirometry measurements with a KoKo spirometer (NSpire Health, Longmont, CO) following the American Thoracic Society guidelines for spirometry performance and interpretation [17].

$^{19}$F and $^1$H MRI procedures

All $^1$H and $^{19}$F MRI were acquired using a Siemens TIM Trio 3 Tesla MRI scanner (Siemens Healthcare, Erlangen, Germany) with multinuclear capabilities. Anatomical $^1$H MR
images for lung mask generation were acquired using a 2D coronal echo-planar fast spin echo sequence (HASTE) with subjects laying feet-first supine (1.3125 mm x 1.3125 mm pixels, 6 mm slice thickness, 1500 ms repetition time, 102 ms echo time, 320 x 320 x 30 acquisition matrix, 150° flip angle, 488 Hz/pixel bandwidth).

¹⁹F MR images were performed using a custom-made 8-channel ¹⁹F-tuned chest coil (ScanMed, Inc., Omaha, NE) tuned to the ¹⁹F frequency. 3D coronal gradient echo images (6.25 mm x 6.25 mm pixels, 15 mm slice thickness, 13 ms TR, 1.63 ms TE, 64 x 64 x 18 acquisition matrix, 90° FA, 130 Hz/pixel bandwidth) were obtained while subjects breathed the exogenous contrast medical grade gas, PFP (79% PFP, 21% O₂; Air Liquide Healthcare, Plumsteadville, PA). Subjects were fitted with a facemask with a non-rebreathing valve that allowed the inhaled gas source to be easily switched between room air and PFP. Subjects were instructed through the breathing cycle; a cycle consisted of two inhaled tidal breaths followed by a full inspiration and a 15-second breath-hold, during which the ¹⁹F MRI was acquired. An initial cycle with room air was completed to set the baseline signal followed by five breathing cycles with the PFP gas to capture ¹⁹F wash-in ventilation. An additional set of at least five cycles (wash-out) were performed until the ¹⁹F signal from the gas was no longer present.

Safety Assessments

Gas flow to the subjects’ breathing masks was monitored using an MR-compatible pneumotachometer (BIOPAC Systems, Inc., Goleta, CA). A finger pulse oximetry system (Model 7500FO; Nonin Medical Inc., Plymouth, MN) acquired heart rate and oxygen saturation levels. To ensure subject safety, outputting Douglas bags were equipped with gas-empty sensors that when triggered, switched the gas supply to room air. Transducer amplifier modules (Model DA 100C; BIOPAC Systems, Inc., Goleta, CA) amplified signals obtained by the pneumotach
transducers. All sampled signals were recorded, digitized and displayed to a PC through a pair of digitizing acquisition modules (Model DI-149; DataQ Instruments, Akron, OH). Windaq (DataQ Instruments, Akron, OH) and Oxigraf (Oxigraf Inc., Mountain View, CA) software allowed for the digital archiving and real-time display of all signals. Exhaled CO$_2$ and O$_2$ levels were recorded utilizing an oxygen gas analyzer (Oxigraf O$_2$Cao; Oxigraf Inc., Mountain View, CA). To confirm that PFP inhalation did not change the lung function of volunteers, spirometry measurements were performed prior to each scan and directly following the imaging session.

*Image Post-Processing*

The anatomical $^1$H MRI and functional $^{19}$F MRI datasets were post processed using MIMVista (MIM Software, Cleveland, OH) and MATLAB (Mathworks, Inc., Natick, MA). $^1$H images were aligned to the $^{19}$F image dataset by means of automatic non-rigid registration transforms. A semi-automatic region-growing algorithm was used to create lung masks based off of the $^{19}$F and conventional $^1$H MRI datasets. Lung masks were utilized to remove background noise outside of the lung field of view (FOV).

Ventilation kinetics within the lung FOV were computed in MATLAB by fitting $^{19}$F signal intensity values to a previously described six-parameter fit model through a Levenberg-Marquardt algorithm[18]. A time series of signal intensity per voxel was created from the temporal compilation of wash-in and wash-out functional $^{19}$F MRI datasets. For a single voxel, the series of signal intensity values over time were fitted to the exponential ventilation wash-in/wash-out model. The model (Equation 1) fits the following parameters: wash-in rate constant
Equation 2. 1. The exponential model equation used to characterize ventilation kinetics from the $^{19}$F MRI datasets.

Time-to-steady-state (TSS) of $^{19}$F signal was defined as the time required to maximum saturation of PFP gas in each voxel as determined from temporally and spatially registered $^{19}$F MR images. A TSS map was produced by finding the difference between $t_1$ and $t_0$ on a voxel by voxel basis.

Ventilation defect volume (VDV) was defined as the volume within the lung field of view (as defined by the conventional $^1$H anatomical MRI) that was not ventilated by the PFP gas, and therefore did not produce a detectable $^{19}$F signal (sum of mean signal intensity of background noise and two times the standard deviation; SD). VDV was expressed as the ratio of thoracic volume calculated from $^{19}$F MR images at maximum wash-in to total thoracic volume measured from the morphological $^1$H MRI.

Each parameter was characterized in terms of estimates of the mean and the standard deviation (SD), with 95% confidence intervals within the lung FOV. Lung function data was compared via Students’ T test.

Results

Six healthy control and eight cystic fibrosis volunteers participated and completed study procedures. Subject characteristics are summarized in Table 1. Data from two subjects were excluded: technical error during the scans resulted in unusable data for the first subject, and a
second data set was excluded from a subject whose beard caused significant gas leakage from the mask. Oxygen saturation remained above 90% in all subjects for the duration of the imaging study, and no adverse effects were observed with the exception of a transient change in voice pitch in two subjects—one healthy control and one CF subject—lasting less than a half-hour after the final PFP gas inhalation.

Table 2. 1. Subject Characteristics

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Healthy</th>
<th>Cystic Fibrosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Subjects</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Female (male)</td>
<td>5 (1)</td>
<td>3 (3)</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>26.7 ± 5.0</td>
<td>33.3 ± 15.1</td>
</tr>
<tr>
<td>Range</td>
<td>21-36</td>
<td>22-65</td>
</tr>
<tr>
<td>Minimum O₂ Levels, %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD Mean</td>
<td>96.2 ± 2.5</td>
<td>96.5 ± 1.8</td>
</tr>
<tr>
<td>Maximum Heart Rate, bpm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>76.8 ± 12.7</td>
<td>89 ± 19.6</td>
</tr>
<tr>
<td>FEV₁ Percent Predicted, %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>105 ± 11</td>
<td>64 ± 24</td>
</tr>
<tr>
<td>Range</td>
<td>89-126</td>
<td>37-105</td>
</tr>
<tr>
<td>FEV₁ Percent Predicted post-imaging, %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>105 ± 12</td>
<td>64 ± 23</td>
</tr>
<tr>
<td>Range</td>
<td>90-129</td>
<td>40-102</td>
</tr>
</tbody>
</table>

Healthy controls had a mean FEV₁ percent predicted of 105% ± 11% (range 89%-126%), while CF subjects had a mean FEV₁ percent predicted of 64% ± 24% (range 37%-105%). Post-imaging session spirometry measurements showed no change in mean FEV₁ measurements in either the healthy control or CF groups following PFP gas inhalation.
In all, 12 complete image datasets were produced; each subject had anatomical $^1$H MR images and functional $^{19}$F MR image sets. Figure 1 displays a typical morphological $^1$H MRI from a healthy control; registration of the anatomical proton MRI with the functional $^{19}$F MRI datasets (Figure 3) aided in the creation of lung masks. Lung masks allowed for the alignment of lung airspace between the anatomic and functional image sets. A series of coronal $^{19}$F MRIs (Figure 2) show the PFP gas airway ventilation in healthy and CF volunteers. Homogeneous PFP airway ventilation was noted in healthy participants, whereas CF subjects—especially those with lower lung function—displayed heterogeneous ventilation. Ventilation defects were also observed in subjects with severe obstruction—in fact, some airway compartments failed to demonstrate any $^{19}$F signal from the PFP gas in spite of the multiple breath protocol.
Figure 2. 2. Coronal images of $^{19}$F MRI scans in a representative normal (top panel) and CF lung with severe obstruction (bottom panel) at end of PFP gas wash-in. Severe obstruction in the CF subject lead to heterogeneous airway ventilation as compared to the healthy control.

Figure 2. 3. Here, an anatomical HASTE $^1$H MRI is overlaid with the functional $^{19}$F MRI dataset (rendered in “hot iron” color scale) to depict the airspace of a healthy control. Registration of the anatomical and functional image sets was completed to assist in the creation of lung masks.

The aligned $^{19}$F MRI time series were used to produce heat maps for each of the six ventilation fit parameters, wherein a single voxel of a parameter map represented the fit parameter value of the corresponding voxel on the anatomical image set. TSS maps for healthy control (FEV$_1$: 3.1 L, 103% predicted) and CF subject with severe disease (FEV$_1$: 1.01L, 37% predicted) are shown in Figure 4. From this figure, the prolonged time to achieve signal equilibrium in the CF lung.
Overall, CF lungs required significantly more time to fill; mean TSS was 324.1 ± 45 seconds and 226.4 ± 33.1 seconds for CF subjects and healthy controls, respectively (p = 0.0021). CF lungs also saw a more heterogeneous filling process, with a skew of -1.8 versus the normal skew of 1.3. Histograms displaying the distribution of individual TSS values throughout the lung airspace were plotted (Figure 5). A threshold TSS value was determined using data from healthy subjects (n=5). The TSS threshold—defined as the sum of the mean of normal TSS values and two SD from the first five normal subjects—was used to classify slow-filling compartments. For CF subjects, 19% ± 22% of the lung volume was defined as slow-filling, whereas only 2% ± 2% of the lung airspace was classified as such in healthy subjects (Satterthwaite t-test p-value = 0.08).
Discussion

Traditionally, the use of MRI techniques to accomplish pulmonary imaging has been far surpassed by the use of MRI on other organs. Recent efforts to develop pulmonary MRI methodologies must overcome intrinsic disadvantages found in the lungs. One such disadvantage is the very short effective transverse relaxation time ($T_2^*$) found in the lungs. This is caused by magnetic field inhomogeneity created by the difference in diamagnetic susceptibility between water in the lungs and air [19]. Low proton density as well as respiratory and cardiac motion artifacts further impede MRI of the lungs [19-21].

Ultra-short echo time (UTE) MRI, described as pulse sequences with echo times (TE) 10-200 times shorter than those conventionally used [22], was developed to combat issues arising from short $T_2^*$ times [22, 23]. Pathophysiologic anatomic variations (bronchiectasis, airway wall thickening, etc.) in young children with CF have successfully been captured with UTE MRI [24]. While shown to correlate well with HRCT in the depiction of anatomical features [23, 24], UTE MRI does not provide functional information such as airway ventilation or perfusion.

Oxygen-enhanced (OE) MRI capitalizes on the shortening of longitudinal relaxation times ($T_1$) in blood, plasma, and tissue in the lungs due to the slightly paramagnetic nature of oxygen [25]. OE MRI involves the inhalation of hyperoxic mixtures of medical-grade oxygen ($\geq 40\%$) during imaging procedures, inherent ventilation and perfusion weighting can then be quantified with parametric maps from the resulting images [25]. Because the kinetics of oxygen transfer can be represented as the magnitude and rate of $T_1$ changes [25], OE $T_1$ mapping has been successfully used to depict lung function. Oxygen-enhanced $T_1$ mapping of lungs in patients with COPD showed that abnormal $T_1$ times positively correlated with perfusion abnormalities [26]. Overall studies indicate that OE MRI correlates well with spirometry measurements, diffusion capacity, and hyperpolarized gas MRI in the detection of ventilation
defects [27]. Notwithstanding, OE MRI abnormalities found in CF patients is likely indirect due to the fact that these abnormalities likely arise from poor gas diffusion [28].

Interest in utilizing hyperpolarized gas MRI has grown in recent years [29-35] as it allows for the depiction of ventilation abnormalities caused by various airway diseases. The necessity of onsite polarizing devices greatly limits gas availability and leads to high costs, furthermore research shows conflicting results regarding the correlation of HP $^3$He MRI ventilation defects to PFTs [32-35]. Performing quantitative ventilation with HP MRI methodologies are further limited by presence of oxygen, which reduces the signal from the hyperpolarized gases [36].

This study explored the early feasibility of performing $^{19}$F MRI in stable CF patients with mild, moderate, and severe lung disease. No adverse effects were observed and sufficient signal was detected in all subjects regardless of disease symptom severity. The data collected from subjects was used to represent ventilation kinetics by modeling the dynamic gas data. Results from this study suggest that patients with CF have VDV reflected in their $^{19}$F MRI datasets, and require significantly increased times to reach $^{19}$F gas ventilation equilibrium than healthy controls. Further studies would be required, however, to ascertain the efficacy of this method in the detection of mild lung disease as compared to other outcome measures. Current data presented in this study is limited by poor understanding of the test-retest variability of $^{19}$F MRI in CF patients. However, $^{19}$F MRI research by Charles et al of asthma patients suggests reasonable short-term repeatability [37]. However, the day-to-day changes in mucus plugging and gas trapping seen in patients with moderate to severe CF could potentially present high retest variability. Notwithstanding, this technique could be invaluable in applications outside of CF. $^{19}$F MRI has already been investigated for other lung disease applications (COPD and asthma)
[13, 15] and it could be useful in other applications such as post-lung transplant surveillance, longitudinal growth in survivors of neonatal respiratory distress. Furthermore, $^{19}$F MRI has the potential to replace low does CT in pediatric CF patients.

The current measure of lung function in this study (TSS) is somewhat limited in the information that it provides. The time required to reach maximum signal places an emphasis on mucosal obstruction and poorly characterizes other pathophysiological features such as bronchiectasis. As an alternative, the wash-in and wash-out rate constants would better characterize these features.

**Conclusion**

F-19 imaging presents a unique opportunity to explore and reduce the heterogeneity of lung function and evaluate treatment response in affected populations without exposing patients to ionizing radiation. Widespread availability of 3T MRI scanners and the commercially available PFP gas makes this technique a relatively feasible option for many academic centers. Unlike other methods of pulmonary imaging (HRCT, UTE MRI, etc.), registration of conventional HASTE sequences with functional $^{19}$F MRI datasets allows for the spatial correlation of structural and functional features for each subject. In the future, ventilation modeling techniques will need to be improved for the incorporation of the wash-in/out rate constants that better represent physiological differences between groups. In addition, further validation of this technique by correlating $^{19}$F MRI ventilation kinetics to other functional imaging modalities will be necessary. $^{19}$F MRI imaging presents a relatively inexpensive, radiation-free method of quantitatively assessing lung function in both healthy and CF populations, creating the potential of an alternative outcome measure for CF clinical trials.
REFERENCES


CHAPTER THREE: FLUORINE-19 MAGNETIC RESONANCE IMAGING FOR CELL TRACKING AND INFLAMMATION IMAGING

Introduction

Cellular-based therapy is a key component in the treatment of a variety of complex medical conditions that require development of noninvasive methods to observe cell behavior and movement within animal and human subjects [1, 2]. Improved understanding of cell bio-distribution also provides information on the development and treatment of many critical diseases, and subsequently their inflammatory responses [1]. One emerging application of fluorine-19 (\(^{19}\text{F}\)) MRI is cell tracking using perfluorocarbon (PFC) probes, a biologically and chemically inert molecule containing \(^{19}\text{F}\) nuclei.

A hallmark of airway disease research has long been the investigation of potential treatment using gene or stem cell therapy [3-7]. Diseases like cystic fibrosis (CF) are characterized by deficient ion transport in columnar epithelial cells, which has a significant clinical impact on the pulmonary system [8]. Consequently, restoration of airway epithelia to address resulting complications (ex. poor mucus clearance, chronic bacterial infection, and inflammation) is of great interest to the CF research community. To address this need, researchers have explored gene and cell therapy as a means to restore function in airway epithelial cells, with varying results [7, 9-11]. In vitro gene therapy studies have shown that even a small percentage of the CF cultured epithelial cells treated with normal CFTR gene vectors can produce healthy phenotypic function [12]. Conversely, localization of therapeutic cells—which are often blood cord- or marrow-derived—to sites of injury in the lungs is not well understood [12]. Current methods used to confirm delivery of stem cells to injured lung tissue
include fluorescence imaging and immuno-histochemical phenotyping [3, 13], both of which are not ideal for animal model studies or clinical trials. $^{19}$F MRI cell tracking techniques can be employed to provide in vivo evidence and quantification of therapeutic stem cells to sites of lung injury in CF models.

Disease models of central nervous system (CNS) disorders such as Alzheimer’s disease (AD) or Parkinson’s disease (PD) are indispensable tools in the search for novel therapies. Recent studies have explored the delivery of therapeutics across the blood-brain barrier (BBB) [14-16]—a major impediment of drug delivery for neurodegenerative disease treatment—to stop and even reverse disease progression in murine models [14, 15]. In contrast, previous PD and AD treatments have focused on providing symptomatic relief at the expense of adverse side effects and patient drug resistance development [17]. Consequently, novel therapies whereby drug-loaded macrophages hone to sites of inflammation in the brain for targeted drug delivery are being explored. Immuncytes like macrophages exhibit diapedesis and chemotaxis processes, which give them an intrinsic homing property of migration to sites of inflammation [18]. Studies have capitalized on this homing property to deliver glial cell-derived neuronal growth factor (GDNF) to produce neuroprotection and reverse the progression in PD mouse models [14]. Though initial investigations of macrophage-mediated drug delivery have shown promising results [14-16], the mechanisms are not fully understood. There is some concern that macrophage loading techniques or inflammation sites aside from CNS inflammation can impact trafficking to the brain [15]. It is therefore necessary to have a means to display and analyze the biodistribution and pharmacokinetics of macrophages in murine models of neurodegenerative disorders.
Cell labeling using PFC probes in conjunction with F-19 MR imaging techniques allows for the visualization of cellular trafficking patterns. Furthermore, inflammation and the inflammatory response, which is associated with cellular trafficking can also be visualized and potentially quantified. Due to the innocuous nature (even at high doses) of PFC probes, $^{19}$F MR cell tracking has utility in providing in vivo information through non-invasive means for various disease models.

Cytotoxicity and loading efficiency studies were conducted on human bronchial epithelial (HBE) progenitor cells—a potential option for future clinical CF cell therapy investigations—and a macrophage-like human monocytic THP-1 cell line. These experiments established labeling procedures and $^{19}$F MRI cell tracking capabilities for future in vivo applications. Cell tracking of inflammatory cells was also conducted on a Parkinson’s disease mouse model using $^{19}$F MRI techniques. F-19 MRI of PFC-labeled murine macrophages was conducted in vivo following toxicity and PFC loading efficiency studies of the macrophages. These studies aim to provide a proof-of-concept for the use of $^{19}$F MRI cell tracking techniques to depict and quantify the in vivo trafficking and biodistribution of PFC-labeled cells in murine models of cystic fibrosis and Parkinson’s disease.

**Methods**

*Cystic Fibrosis Model Cell Culture Procedures*

Experiments were performed using HBE stem cells, harvested from the excised lung tissue of healthy adults (n=3) and purchased macrophage-like human monocytic THP-1 cell line (ATCC TIB-202; American Tissue Culture Collection, Manassas, VA) previously described [19, 20].

HBE progenitor cells were cocultured with irradiated fibroblasts to create conditionally reprogramed cell (CRC) which conditionally induce cell proliferation.
Irradiated 3T3-J2 mouse fibroblasts were cultured at 37°C and 5% CO<sub>2</sub> in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum, 100 μg/mL penicillin, 100 μg/mL streptomycin, and 100 μg/mL glutamine (complete DMEM; Gibco, Gaithersburg, MD). After allowing 3T3 J2 murine fibroblasts feeders to attach for 24 hours, CRC cultures were co-seeded on a feeder layer in F +Y medium and passaged every 3-5 days. At 80% confluence, feeders were removed via brief Accutase (STEMCELL Technologies, Inc., Vancouver, Canada) incubation and gentle agitation to prepare HBEs for further use in experiments.

Human monocytic THP-1 cells were cultured in RPMI-1640 media (RPMI Media 1640; Gibco, Gaithersburg, MD) containing 10% fetal bovine serum (FBS), 1.4 mmol/L L-glutamine (Gibco, Gaithersburg, MD), 50 μg/mL penicillin, 50 μg/mL streptomycin, and 100 μg/mL neomycin (Gibco, Gaithersburg, MD) at 37°C and 5% CO<sub>2</sub>. THP-1 cells were differentiated from monocytes to macrophages by incubation in 200 nM phorbol 12-myristate 13-acetate (PMA; Sigma, St. Louis, MO) for a period of 72 hours.

**Parkinson’s Disease Model Cell Culture Procedures**

Macrophages extracted from the bone marrow of mice were obtained from the femurs of C57/BL male mice as previously described [21]. Cells were cultured for 12-14 day in DMEM (DMEM; Invitrogen, Carlsbad, CA) medium supplemented with 100 U/mL macrophage colony-stimulating factor (MCSF). Murine macrophages were labeled with Cell Sense at concentrations of 0 mg/mL (unlabeled negative control), 0.75 mg/mL, or 1.5 mg/mL for 4- or 24- hour periods.

**Animals**

A Parkinson’s disease mouse model was created by inducing inflammation of the striatum (SN) in two C57BL/6 mice using a 1μL lipopolysaccharide (LPS) injection. Bone
marrow-derived macrophages (BMM) labeled at a concentration of 1.5 mg/mL of Cell Sense for 24 hours were introduced 24 hours after LPS injections through intravenous (IV; $2 \times 10^6$ cells) or intraventricular (Ivt; $2 \times 10^5$ cells) injections.

This study was performed in accordance with the recommendations of *Principles of Animal Care* outlined by the National Institutes of Health and approved by the Institutional Animal Care and Use Committee of the University of North Carolina at Chapel Hill. Surgeries were performed under sodium pentobarbital anesthesia to minimize animal suffering. All manipulations were carried out under a sterilized laminar hood, and food and water were given *ad libitum*. Mice were anesthetized with 1% isoflurane gas for the duration of imaging procedures.

**Cytotoxicity and Fluorescence Measurements**

Following removal of feeders, CRC-grown HBE cultures were seeded onto a collagen coated black, flat glass bottom 96-well plate (Corning Inc., Durham, NC) at a density of $10^4$ cells per well ($n=3$). CRC-grown HBEs were incubated at 37°C and 5% CO$_2$ in F+Y media for 24 hours to maximize adherence. THP-1 cells and murine macrophages were also seeded onto a collagen coated black, flat glass bottom 96-well plate at a density of $10^4$ cells per well and differentiated as described above. All cell types were washed with PBS and labeled with a dual $^{19}$F MRI and fluorescence imaging agent, Cell Sense (CS-ATM DM Red, Celsense, Inc., Pittsburgh, PA). Cell Sense is a perfluoropolyether (PFPE) nanoemulsion with a total fluorine content of 120 mg/mL designed to facilitate cellular uptake across all cell types. Labeling was achieved by incubating cells for 24- and 48-hour periods in their respective media containing either 0 mg/mL (negative control), 1.5 mg/mL, 3.0 mg/mL, 6.0 mg/mL or 12.0 mg/mL Cell Sense for the HBEs and THP-1 macrophage-like cells. Murine C12 macrophages were
incubated for 4- and 24-hour periods in media containing either 0 mg/mL, 0.75 mg/mL, or 1.5 mg/mL Cell Sense.

Cells were washed three times with PBS following incubation in Cell Sense and 100 μL of their respective media was added to each well for fluorescence imaging, quantitative fluorescence measurements and a cytotoxicity assay. Plates were loaded into an Infinite M1000 plate reader (TECAN, Männedorf, Switzerland). Fluorescence was measured with an excitation and emission wavelength of 595 nm and 610 nm, respectively. Cytotoxicity was evaluated via a water-soluble tetrazolium salt assay (WST-1 Cell Proliferation Assay System; Takara Bio Inc., Shiga, Japan) according to the manufacturer protocol. Absorbance was measured using a Cytation 5 (Biotek, Winooski, VT, USA) cell imaging multi-mode reader at an emission wavelength of 440 nm.

**NMR Spectroscopy**

Cultured cells were labeled with Cell Sense and incubated in standard conditions for 24 hours at concentrations of 0 mg/mL, 1.5 mg/mL or 3.0 mg/mL. Cells were washed three times with PBS and pelleted via centrifugation for NMR experiments.

One-dimensional $^{19}$F NMR spectra were recorded with a Varian Inova 500 MHz spectrometer (UNC Eshelman School of Pharmacy NMR Facility, Chapel Hill, NC) at ambient temperature with reference to trifluoroacetic acid (TFA; Aldrich Chemical Co., Milwaukee, WI) and deuterium oxide (D$_2$O; Aldrich Chemical Co., Milwaukee, WI) as the solvent. Data was analyzed using the Varian built-in software, VNMR (VNMR Version 6.1; Varian, Inc., Palo Alto, CA).
**MR and Fluorescence Imaging**

Fluorescence imaging was performed on an Olympus IX-81 inverted wide-field microscope (Hooker Imaging Core, UNC, Chapel Hill, NC).

Conventional $^1$H MRI and $^{19}$F MRI were acquired on a horizontal 9.4 Tesla Bruker Biospec Small Animal Scanner (UNC Small Animal Imaging Core, Chapel Hill, NC). The MRI scanner was equipped with a custom-made dual-tuned $^1$H/$^{19}$F surface coil with a 2.5 cm diameter. $^{19}$F MR images were acquired using two-dimensional rapid acquisition with relaxation enhanced (2D RARE) sequence with a RARE factor of 32, repetition time (TR) = 1000 ms, echo time (TE) = 8.765 ms, 3000 signal averages, a 10kHz bandwidth, field-of-view (FOV) of 3.2 cm x 3.2 cm and an image matrix size of 32 x 32. Reference phantoms with a known quantity of F atoms was situated within the FOV for cell quantification purposes (300 μL 0.75 Cell Sense and 300 μL 1.5 Cell

![Fluorescence images of labeled THP-1 cells (left) and CRC-grown HBEs (right) taken following a 24-hour incubation period in media containing shown Cell Sense concentrations.](image)
Sense). HBE progenitor cells labeled in 3.0 mg/mL Cell Sense for 24 hours were used to prepare cell phantoms with $1 \times 10^6$ and $4 \times 10^6$ cells, respectively.

Results

Cystic Fibrosis Model Results

Both the HBEs and macrophage-like THP-1 cells were loaded with the PFC probe, Cell Sense. Inverted microscope images of both cell types treated with varying labeling concentrations of Cell Sense were captured following a 24-hour incubation period. Images (Figure 3.1) show increasing fluorescence with increasing concentrations of Cell Sense in the incubation media.

The increase in fluorescence with PFC probe concentration is also reflected in TECAN plate reader measurements (Figure 3.2). While an ANOVA test revealed that the difference in mean fluorescence was statistically significant ($p<0.05$) between labeling conditions for both cell types, pairwise t-tests indicated that only THP-1 cells incubated at a Cell Sense concentration of 12.0 mg/mL fluoresced more than THP-1 cells incubated at lower concentrations. For HBEs however, pairwise t-tests showed that the difference in fluorescence was statistically significant between all concentration levels with the exception of one; the difference in fluorescence was not statistically significant between cells incubated at 1.5 mg/mL and 3.0 mg/mL (Table 3.1). In essence, higher labeling concentrations resulted in increased Cell Sense uptake, but not always significantly so.
Table 3.1 Pairwise t-test p-values of fluorescence between labeling conditions for THP-1 and HBE cells. P-values greater than 0.05 indicates a statistically insignificant difference in fluorescence between the two Cell Sense labeling concentrations.

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Cell Sense Concentration</th>
<th>1.5 mg/mL</th>
<th>3.0 mg/mL</th>
<th>6.0 mg/mL</th>
<th>12.0 mg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>THP-1 macrophage-like</td>
<td>0 mg/mL</td>
<td>p &gt; 0.05</td>
<td>p &gt; 0.05</td>
<td>p &gt; 0.05</td>
<td>p = 3.2e-7</td>
</tr>
<tr>
<td></td>
<td>1.5 mg/mL</td>
<td>-</td>
<td>p &gt; 0.05</td>
<td>p &gt; 0.05</td>
<td>p = 4.0e-7</td>
</tr>
<tr>
<td></td>
<td>3.0 mg/mL</td>
<td>-</td>
<td>-</td>
<td>p &gt; 0.05</td>
<td>p = 7.5e-7</td>
</tr>
<tr>
<td></td>
<td>6.0 mg/mL</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>p = 4.6e-6</td>
</tr>
<tr>
<td>CRC-grown HBE</td>
<td>0 mg/mL</td>
<td>p = 2.7e-9</td>
<td>p = 4.3e-12</td>
<td>p &lt; 2e-16</td>
<td>p &lt; 2e-16</td>
</tr>
<tr>
<td></td>
<td>1.5 mg/mL</td>
<td>-</td>
<td>p &gt; 0.05</td>
<td>p = 2.9e-8</td>
<td>p &lt; 2e-16</td>
</tr>
<tr>
<td></td>
<td>3.0 mg/mL</td>
<td>-</td>
<td>-</td>
<td>p = 3.2e-5</td>
<td>p = 1.2e-15</td>
</tr>
<tr>
<td></td>
<td>6.0 mg/mL</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>p = 3.6e-9</td>
</tr>
</tbody>
</table>

WST-1 assay toxicity experiments showed little difference in cell viability based on concentration of Cell Sense present in incubation media; ANOVA tests revealed that differences between labeling conditions were not statistically significant for either cell type (p > 0.05). As compared to negative controls (unlabeled cells), PFPE-labeled cells exhibited at least 80% survival (Figure 3.3).
Figure 3.5. WST-1 assay toxicity studies demonstrated that incubation of cell in the labeling agent had little impact on survivability.

NMR studies were performed on THP-1 macrophage-like and HBE cell pellets (n=3 for each cell type and labeling concentration) incubated in media containing 1.5 mg/mL and 3.0 mg/mL Cell Sense for 24 hours (Figure 3.4). Table 3.2 summarizes the mean loading efficiency for all cell types and labeling conditions.

Table 3.2. Loading efficiency (fluorine atoms per cell) of the PFC probe were determined based on NMR spectra peaks (shown in Figure 3.4) of the cell pellets relative to a TFA reference.

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Estimated F/cell</th>
<th>Cell Sense Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>THP-1 macrophage-like (n = 3)</td>
<td></td>
<td>1.5 mg/mL</td>
</tr>
<tr>
<td></td>
<td>9.84 x 10^{12}</td>
<td>3.0 mg/mL</td>
</tr>
<tr>
<td>CRC-grown HBE (n = 3)</td>
<td>1.24 x 10^{13}</td>
<td></td>
</tr>
<tr>
<td>Murine BMM (n = 2)</td>
<td>9.20 x 10^{13}</td>
<td></td>
</tr>
</tbody>
</table>
Figure 3.5a shows HBE labeled cell pellets (1 x 10^6 and 4 x 10^6), that were imaged approximately 24 hours after washing using \(^{19}\)F MRI techniques. Figure 3.5b shows the correlation between cell number per mm\(^3\)-voxel and normalized signal-to-noise ratio (SNR) for each cell phantom. An estimate for the minimum number of HBE progenitor cells per mm\(^3\)-voxel needed to produce a visible signal—4.0 x 10^3 cells/voxel—was estimated from the correlation between cell quantity and SNR.

Figure 3.6. Cells are efficiently labeled with the PFC probe ex vivo. \(^{19}\)F NMR spectra for pelleted and labeled (3 x 10^6 cells, 1.5 mg/mL Cell Sense for 24 hours) (a) macrophage-like THP-1 cells, (b) CRC-grown HBEs, and (c) murine BMMs.
Figure 3. $^{19}$F MRI Phantom studies (a) were acquired using previously outlined imaging parameters on two cell phantoms containing $1 \times 10^6$ and $4 \times 10^6$ cells, respectively. The “R” designated the Cell Sense reference phantom; (b) Signal normalized to a 1 mm$^3$-voxel was calculated from $^{19}$F MRI and correlated to cell quantity per voxel for cell quantification purposes. Cell quantification was validated by (c) the actual versus quantified cell number per voxel. The Pearson correlation coefficient is 0.99.

**Parkinson’s Disease Mouse Model Results**

Cell viability and PFC probe loading efficiency studies were conducted on murine BMM. Toxicity experiments (Figure 3. 6) showed some impact on cell survival rate based on Cell Sense concentration levels in incubation media. ANOVA analysis revealed a significant difference in toxicity due to differing Cell Sense concentrations ($p = 0.00252$), and subsequent pairwise t-tests determined that only cells incubated in 3.0 mg/mL Cell Sense experienced significantly lower survival rates. $^{19}$F NMR analysis of pelleted labeled BMM (Figure 3. 4c) was used to determine cell PFC-loading efficiency and validate cellular uptake of Cell Sense (Table 3. 2).
Ex vivo PFC-labeled cells were injected into mice either intravenously (2 x 10^6 cell/200 μL) or intraventricularly (2 x 10^5 cell/5 μL). Figures 3. 7 a and 3. 7 d show the proton images of axial slices for each mouse, acquired 24 hours after injection (day 1). Corresponding ¹⁹F images are shown in Figures 3. 7 b and 3. 7 e, where only the reference phantom and injected cells were detectable. The overlay between the proton and fluorine images are show in Figures 3. 7 c and 3. 7 f, allowing for localization of injected cells in the mouse body.

In vivo ¹⁹F signal was tracked over time, yielding a decrease over time in the total estimated number of cells present intracranially (Table 3. 3).
Table 3. The number of labeled BMMs present in mice was quantified over time in each mouse.

<table>
<thead>
<tr>
<th>Estimated Number of Cells (intracranial)</th>
<th>Intraventricular Cell Injection</th>
<th>Intravenous Cell Injection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 hours post-injection (day 0)</td>
<td>24 hours post-injection (day 1)</td>
</tr>
<tr>
<td></td>
<td>2.7 x 10^5</td>
<td>1.3 x 10^5</td>
</tr>
<tr>
<td>Estimated Number of Cells (extracranial)</td>
<td>-</td>
<td>1.4 x 10^5</td>
</tr>
<tr>
<td>Total Estimated Number of Cells</td>
<td>2.7 x 10^5</td>
<td>2.7 x 10^5</td>
</tr>
</tbody>
</table>

Figure 3. Representative axial proton (a and d), fluorine (b and e), and overlay (c and f) images with ^19^F signal rendered in “hot metal” color scale on the day after cell injection. The green arrows point to intracranial Cell Sense-labeled BMM, while extracranial murine macrophages are demarcated by yellow arrows. “R” designates the reference phantom. The mouse depicted in a though c received 2 x 10^5 cell intraventricularly and was imaged 24 hours after the introduction of cells. The lower panel (d through f) shows a mouse that received 2 x 10^6 cells intravenously and was imaged 24 hours after the cell injection.
Discussion

These studies evince efficient labeling of multiple cell types with a PFC probe with some effect on cell viability. These results are supported by previous investigations [22-24] that show the ability of PFPE-marker loading with minimal impact on cytotoxicity. $^{19}$F MRI signal detectability was demonstrated in vitro and in vivo using Cell Sense, an FDA-approved cellular imaging agent. An alternative cell tracking method is superparamagnetic iron-oxide (SPIO) MRI, wherein SPIO nanoparticles serve as contrast labeling agent for cells as a method to exploit the advantages of MRI for cell tracking [24-28]. Cells labeled ex vivo or in situ with SPIO experience a significant shortening of $T_1$ and $T_2$ relaxations times of those cells relative to their environment, thus allowing for the observation cellular contrast changes by MR imaging [26]. While metal-ion cell labeling offers increased sensitivity, its drawbacks include complex cell number quantification and biocompatibility issues [24, 25]. Furthermore, as a negative contrast technique, its application in the high susceptibility region such as the lung, is limited. Current methods of imaging inflammation and the inflammatory response are limited to anatomical images of tissue to capture visible changes [29]. Conventional imaging modalities are limited in their ability to visualize inflammation by spatial resolution and tissue penetration depth. Consequently, inflammation imaging by anatomical methods often provides information in the late stages of inflammation—when the damage often becomes permanent [1, 29]. PFC labeling of inflammatory cells allows for the visualization and quantification of inflammatory cells, providing researchers with information on the early inflammatory response. Early imaging of inflammation is the gateway for diagnostic inflammation markers and the quantification of inflammation in subjects over time [1]. This is the first study to apply $^{19}$F MRI techniques for inflammation imaging and cell tracking purposes in CF and PD models.
Cytotoxicity, Labeling Efficiency, and $^{19}$F MRI cell detection for CF models

Fluorescence imaging, fluorescence measurements, and $^{19}$F NMR demonstrated the uptake of Cell Sense by THP-1 macrophage-like cells and CRC-grown HBE progenitor cells, with little impact on cell viability. Due to the phagocytic behavior of macrophages, a higher labeling efficiency was expected for THP-1s versus HBEs, however $^{19}$F NMR uptake validation studies revealed that this was not the case for the one (1.5 mg/mL, 24-hour incubation) labeling condition.

As HBEs are a likely candidate for future cell therapy investigations, this study focused on the determination of SNR limits for PFC-loaded HBE progenitor cells. In vitro $^{19}$F MRI of labeled HBEs demonstrated the capability to image at least $4.0 \times 10^3$ cells/voxel to provide visible signal. Current imaging parameters may prove to be a limitation for future studies, which require in vivo imaging of labeled therapeutic bronchial epithelial cells engrafted onto injured mice tracheas and other levels of the airway tree. Such studies would likely not involve such high labeled-cell counts in murine models. Future studies may require the use of alternative MRI sequences more optimized for $^{19}$F MRI cell tracing. Notwithstanding, fluorine imaging methods in this study proved to be capable of cell quantification in vitro. The HBE cell quantification experiments showed a strong correlation between measured cells/voxel and actual cells/voxel.

$^{19}$F MRI in a PD murine model

$^{19}$F NMR studies demonstrated cellular uptake of the Cell Sense labeling agent. Unlike the THP-1 macrophages, the murine bone marrow-derived macrophages higher labeling concentrations negatively impacted viability, necessitating the use of a more dilute labeling condition for further studies (1.5 mg/mL, 24-hour incubation). This difference in viability can potentially be attributed to the $\approx$7-fold and $\approx$9-fold increase in loading efficiency by BMM as compared to HBE progenitor cells and THP-1 macrophages, respectively.
This study demonstrated in vivo 19F MRI inflammation imaging of labeled BMMs in murine PD models, and sufficient SNR was achieved for the detection of PFPE-labeled cell by 19F MRI. Both mice were imaged at the time point when LPS-induced inflammation was anticipated to be the greatest (48 hours after LPS injection, i.e. 24 hours after PFC-labeled cells were introduced). Additional time points were explored for the mouse that received an intraventricular labeled-cell injection to allow for cell tracking over time. These results show that the number of labeled cells decreased overtime, with the exception of extracranial cells from day 0 to day 1 (Table 3. 3). As labeled cells migrated out of the brain— and presumably entered circulation— on the day of labeled cell injection, the fluorine signal originating outside of brain space increased. Additional labeled-cell delivery models as well as an increase in sample size will be necessary for future studies. These results act as a proof-of-concept, and further 19F MRI cell tracking investigations with a greater number of mice, experimental and control conditions is necessary.

**Conclusion**

Moving forward, it will be necessary to conduct further investigations to further substantiate the use of 19F MRI for cell tracking and inflammation imaging in small animal models. Confirmation of targeted delivery of the therapeutics to the desired site will through alternative more established means (ex. fluorescence imaging of fixed histological slides) will be necessary.

Studies conducted in this chapter have shown that 19F MRI methods can be employed to detect and track fluorine-labeled cells in two different murine disease models. These studies demonstrated the proof-of-concept for the application of 19F MRI in in vivo cell tracking and inflammation imaging, which are critical tools for cell therapy research in disease models where inflammatory response impact disease progression. While continuing investigations are
necessary to improve this technique, thus far it is clear that $^{19}$F MRI provides the ability to study cell biodistribution and inflammatory response using preclinical small animal models at high magnetic fields.
REFERENCES


CHAPTER FOUR: CONCLUSION

The investigations included in this thesis incorporate the implementation of $^{19}$F MRI techniques to achieve their respective goals of human pulmonary ventilation imaging and in vivo cell tracking and inflammation imaging. The ability to perform $^{19}$F MRI relied heavily on applying conventional proton MRI techniques in a multinuclear setting. This was feasible at a relatively low cost because $^1$H and $^{19}$F isotopes have resonances within 6% of each other. Existing clinical scanners are easily equipped with $^{19}$F or dual-tune $^1$H/$^{19}$F coils to perform $^{19}$F magnetic resonance imaging. Additionally, commercially available fluorinated compounds are widely available. Compounds such as PFP and Cell Sense have been investigated for a number of years and are well characterized for in vivo use in humans and animals commonly used in disease modeling [1-4]. These compounds demonstrated the ability to function as chemically inert, non-toxic $^{19}$F contrast agents in a number of applications.

$^{19}$F MRI of pulmonary ventilation in healthy human volunteers and volunteers with CF experiments illustrated the potential to use this technique for functional imaging of the lungs. Similarly, the cell tracking studies explored here established the feasibility and utility of this technique for use in an in vivo CF and PD mouse model. Future development of pulse sequences will improve the use of $^{19}$F for these applications, especially for ventilation imaging where current image acquisition requires breath-holds. Additionally, ongoing development in the improvement of labeling agents will further improve SNR and reduce image acquisition times for in cell tracking experiments.
Overall, these studies established that $^{19}$F MRI techniques can perform pulmonary ventilation imaging and *in vivo* cell tracking and inflammation imaging in humans and small animals.
REFERENCES


