Published in final edited form as:

Magn Reson Med. 2013 July ; 70(1): . doi:10.1002/mrm.24436.

Rapid quantification of oxygen tension in blood flow with a fluorine nanoparticle reporter and a novel Blood flow-Enhanced-Saturation-Recovery (BESR) sequence

Lingzhi Hu¹, Junjie Chen², Xiaoxia Yang², Shelton D. Caruthers², Gregory M. Lanza², and Samuel A. Wickline^{1,2,*}

¹Department of Physics, Washington University in St. Louis, MO, 63110, USA

²Department of Medicine, Washington University in St. Louis, MO, 63110, USA

Abstract

We present a novel Blood flow-Enhanced-Saturation-Recovery (BESR) sequence, which allows rapid in vivo T1 measurement of blood for both ¹H and ¹⁹F nuclei. BESR sequence is achieved by combining homogeneous spin preparation and time-of-flight image acquisition and therefore preserves high time efficiency and SNR for ¹⁹F imaging of circulating Perfluorocarbon (PFC) Nanoparticles (NPs) comprising a perfluoro-15-crown-5-ether core and a lipid monolayer (nominal size = 250 nm). The consistency and accuracy of the BESR sequence for measuring T1 of blood was validated experimentally. With a confirmed linear response feature of ¹⁹F R1 with oxygen tension in both salt solution and blood sample, we demonstrated the feasibility of the BESR sequence to quantitatively determine the oxygen tension within mouse left and right ventricles under both normoxia and hyperoxia conditions. Thus, ¹⁹F BESR MRI of circulating PFC NPs represents a new approach to non-invasively evaluate intravascular oxygen tension.

Keywords

perfluorocarbon nanoparticle; oxygen tension; blood flow; T1 measurement; cardiac MRI; pulse sequence

Introduction

Blood oxygen tension (pO₂) is an essential pathophysiological index of circulatory and respiratory health that is useful for characterizing various disorders such as congenital heart disease (1), arteriovenous malformations (2), atherosclerotic dysfunction (3), and overt or occult heart failure in general (4). The current gold standard for pO₂ measurement entails direct blood sampling using arterial puncture, or invasive catheterization when A-V O₂ differences, or cardiac output and oxygen consumption data are needed.

MRI has been exploited as an alternative tool for non-invasive assessment of blood oxygenation based on blood-oxygenation-level-dependant (BOLD) MRI determined ¹H R2* (5), susceptibility (6), or T2 and T1 contrast (7-8). Among these methods, R2* and susceptibility contrast generated by deoxygenated-hemoglobin (deoxy-Hb) can be used to estimate deoxy-Hb concentration and blood saturation (SO₂) because deoxy-Hb is highly paramagnetic. Nevertheless, the readout of BOLD and susceptibility-dependent contrast is

*Send correspondence to: Samuel A. Wickline, C-TRAIN Group, Campus Box 8215, Washington University School of Medicine, Cortex Building, Suite 101, 4444 Forest Park Ave, St. Louis, MO 63108, USA, Phone: 314-454-8635, Fax: 314-454-5265, saw@wuphysics.wustl.edu.

qualitative, or otherwise requires organ-specific prior knowledge to build complex mathematical models (5-6). Blood flow imaging with global T2 preparation has also been employed to measure blood oxygenation because of its linear correlation with blood ^1H R2. However, for in vivo studies, B_1 and B_0 inhomogeneity and pulsatile flow artifacts pose major obstacles for effective spin preparation and consistent R2 estimation (8). Additionally, blood ^1H R1 is also linearly responsive to dissolved pO_2 and thus may also be employed as a quantitative marker for blood oxygenation. However, because of the limited solubility of O_2 in plasma, the slope of ^1H R1 as a function of pO_2 is as low as $2 \times 10^{-4} \text{ s}^{-1}/\text{mmHg}$, resulting in significant uncertainty for in vivo pO_2 quantification (7).

Perfluorocarbon nanoparticles (PFC NPs) have been proposed as an ^{19}F MR pO_2 sensor (9-10) as a consequence of two attributes: (a) O_2 is a weakly paramagnetic molecule with unpaired electrons and net magnetization; (b) O_2 exhibits high solubility in PFC; (c) the linear O_2 release response curve of PFC and the free O_2 diffusion between PFC and surrounding environment render PFC as a reliable pO_2 probe for in vivo applications of measuring vascular pO_2 (11-12) and tissue oxygenation (9,13-14). Previous reports have demonstrated that the slope of ^{19}F R1 of PFC as a function of pO_2 could be ten-fold greater than the dependence of ^1H R1 on pO_2 (12-13,15). Additionally, because the lipid monolayer of PFC NPs is impermeable to macromolecules and ions, free paramagnetic macromolecules and ions in the blood stream do not affect the measurement of ^{19}F R1 emanating from PFC NPs, or its dependency on pO_2 (16). Finally, as a FDA-approved oxygen delivery vehicle, PFC NPs exhibit an acceptable bio-safety profile for ultimate clinical use (17-18).

Similar to ^1H MRI (19-22), one major obstacle of intravascular pO_2 measurements based on ^{19}F T1 of PFC NPs is the blood pulsatile motion artifact and in-flow effect. Other limitations include relatively low signal-to-noise ratio (SNR) due to limited amount of injected PFC NPs in the blood pool, and the inhomogeneity of spin preparation due to the movement of arterial blood through a large portion of body during the relaxation measurement. Therefore, the implementation of novel ^{19}F MRI strategies to minimize these artifacts would be critical to blood pO_2 measurements obtained by ^{19}F MRI relaxometry.

In the present work, we have developed a novel Blood flow-Enhanced-Saturation-Recovery (BESR) sequence for measuring ^{19}F T1 of flowing PFC NPs in blood pool. The BESR sequence preserves the time efficiency of the classical Look-Locker sequence and the high SNR of saturation recovery sequence. We first performed a computer simulation to illustrate the homogeneity of spin preparation in the BESR sequence, and to define the effect of limited SNR on the precision of the pO_2 measurement. The accuracy of BESR sequence measured T1 was tested on a phantom and evaluated in vivo by measuring arterial ^1H T1 in carotid arteries and cardiac ventricles. Finally, we optimized scan times and PFC NPs doses in conjunction with the BESR sequence for ^{19}F imaging of circulating PFC NPs to provide a quantitative estimate of arterial and venous pO_2 in mouse heart ventricles under both normoxia and hyperoxia conditions.

Methods

The BESR sequence was implemented and all images were acquired on a 4.7 T Varian INNOVA scanner. All procedures in this study conformed to the guidelines and approvals by the Animal Studies Committee of Washington University in St. Louis.

Pulse Sequence and Imaging Setup

The BESR pulse sequence is designed for measurement of pO_2 in pulsatile blood flow. It comprises two parts: homogeneous spin preparation and time-of-flight image acquisition (Fig. 1). The spin preparation is achieved with a non-selective whole body saturation pulse

train and the image acquisition is realized with gradient echoes at high repetition rate. Both the pre-pulses and imaging excitation pulses are gated to the ECG with appropriate trigger delay to minimize the motion artifact associating with the beating heart and vascular contraction and dilation.

In contrast to measuring the T1 of solid tissues, the spin preparation for measuring T1 of flowing blood should be non-selective and homogenous over the entire subject because spins in the blood that will be excited for imaging might arise from a variety of locations during the spin preparation step (22-23). To achieve homogeneous spin preparation, an actively-decoupled transmit-only saddle coil (O.D. = 7cm, length = 12 cm) that covers the entire mouse body and a composite RF saturation pulse train comprising five constitutive 90° sinc-shaped pulses are employed. The bandwidth of each saturation pulse is 12 KHz and the time delay between two adjacent saturation pulses is 2 ms, during which crusher gradients along different directions are applied. The homogeneity of spin preparation is expected to improve progressively with the number of 90° pulses in the saturation pulse train. Specifically, $s(x, y, z)$, the percentage residual magnetization along z direction in a voxel positioned at (x, y, z) after n closely spaced saturation pulses followed by crusher gradients can be written as

$$s(x, y, z) = [\cos(\phi(x, y, z))]^n, \quad [1]$$

where $\phi(x, y, z)$ is the flip angle at (x, y, z) and is determined by the B_1 field profile according to $\phi(x, y, z) = B_1(x, y, z) \tau \gamma$, where γ is the Larmor frequency and τ is the pulse width. To confirm the homogenous saturation over the region of interest (ROI), we first acquired a flip angle mapping of our transmit coil on a water phantom (D = 6 cm, length = 10 cm) using a dual angle B1 mapping approach with $\alpha = 30^\circ$ (24). Quantitative residual magnetization mapping after various number of saturation pulses (NSP) was then obtained by adjusting NSP in the BESR sequence.

In traditional ^1H saturation recovery T1 mapping, only one imaging excitation pulse is allowed within in a single TR to avoid partial saturation effects arising from frequent repetitive excitations (25). In contrast, because of relatively fast blood flow in major blood vessels (>1 cm/s) and the “time-of-flight” effect, we anticipate that excited spins in the previous echo will flow out of the imaging slice as long as the time interval between two temporally adjacent excitation pulses is sufficiently long (e.g., >0.2 s for 2 mm slice). Within a single TR of the BESR sequence, multiple 90° excitations, each followed by a same phase encoding gradient, are applied for gradient echo imaging. Thus, all echoes acquired in a single TR correspond to the same k-space line at different saturation delays (TS). All the imaging excitation pulses are synchronized with the ECG to minimize the effect of heart rate variations during imaging (26). Finally, to achieve an optimal SNR with BESR sequence, a dedicated actively-decoupled receive-only surface coil (diameter $\approx$ 3 cm) is placed close to the region of interest for signal reception.

Estimating the effect of SNR on the accuracy of pO₂ measurement

The uncertainty of the pO₂ estimation caused by random noise in saturation recovery imaging was evaluated using computer simulations in Matlab (Mathworks, USA) and conventional saturation recovery imaging on PFC NPs phantoms. A region of interest (ROI) containing 100 pixels was defined to simulate the number of pixels in heart ventricles. Normally distributed Gaussian noise was imposed on the ^{19}F signal intensity in each pixel to achieve variable SNR. The average noise-contaminated signal in entire ROI was used for non-linear fitting of T1 values. The conversion between T1 and pO₂ values was based on the pre-acquired calibration curve. In the simulation, the following saturation delays were used: TS = 0.25, 0.5, 0.75, 1, 1.25, 1.5, 1.75, 2 and 10 s. Simulation was repeated 10000 times and

the standard deviation of the fitted pO_2 was calculated to represent the measurement uncertainty propagated from imaging noise through non-linear fitting. For phantom imaging, three PFC NPs phantoms with volume concentration of 5%, 10% and 20% were sequentially bubbled with N_2 , room air and O_2 for 10 minutes. The phantom temperature was maintained at $37^\circ C$ with circulating water bath. A saturation recovery fast spin echo sequence was used for T1 mapping: TR=3 s, FOV=25 cm $\times$ 25 cm, TE = 8.6 ms, echo train length = 16, Matrix size=128 $\times$ 128. The standard deviation of the fitted pO_2 values for each single pixel was used to calculate the uncertainty of pO_2 estimate in an ROI containing 100 pixels.

In vitro and in vivo validation of BESR sequence

The accuracy and precision of the BESR sequence was tested by measuring 1H T1 of circulating water in a tube phantom, which simulated the in vivo setting of ^{19}F MRI with all signal contributed only by intravascular PFC NPs devoid of tissue background. Briefly, approximately 80 cm silicon tubing (1/8" inner diameter) was twisted and coiled to form an 8-cm long phantom with multiple loops. The phantom was then connected to a pump to generate constant 5 cm/s flow velocity to mimic blood flow. The special design of the phantom together with the carefully chosen flow velocity simulate the blood flow situation in vivo, such that the flowing water could experience a homogenous spin preparation at the beginning of each TR (5 s), but is moving rapidly enough to generate the desired "time-of-flight" effect to replenish the imaging slice with unexcited spins before each gradient-echo (10 echoes in each TR ranging from 0.1 s to 4.6 s time interval between consecutive imaging acquisitions = 0.5 s). Upon the completion of BESR measurement, the pump was turned off and the 1H T1 of stationary water was measured with a saturation recovery fast spin echo sequence. The image parameters of fast spin echo were TR=5 s, TE = 8.6 ms, echo train length =16, TS=0.1 to 4.6 s with intervals of 0.5 s.

To confirm that the BESR sequence can achieve reproducible T1 measurements in vivo, 1H T1 measurements of flowing arterial blood in carotid arteries and heart ventricles were acquired in Swiss Webster mice (n=5) at 4.7 T. Mice were anesthetized through inhalation of 1.5% isoflurane ventilated by pure O_2 (2 L/min). Body temperature was maintained at $37^\circ C$ and ECG was monitored with a small animal monitor system (SAI Inc., USA). During MRI, the isoflurane level was adjusted to maintain mouse heart rate at around 350 bpm. Imaging parameters were: TR=2.5 s, TE = 2.8 ms, FOV=25 cm $\times$ 25 cm, matrix size=96 $\times$ 96 and number of TS=7. After in vivo imaging, mice were sacrificed and blood was collected from LV. T1 of the collected stationary blood was measured using a custom-built solenoid coil and traditional inversion recovery spectroscopy at $37^\circ C$ after saturating with pure O_2 .

PFC NP formulation and in vitro T1 calibration

Perfluoro-15-crown-5-ether (CE) nanoparticles were formulated as previously described. Briefly, the CE emulsion was composed of 40% (v/v) of CE (Exfluor Research Corp., USA), 2.0% (w/v) of a surfactant commixture, and 1.7% (w/v) glycerin, with water comprising the balance. In vitro calibration of ^{19}F R1 as a function of pO_2 was performed at three different O_2 concentrations (0%, 21%, and 100% O_2 balanced with N_2) by bubbling gas mixture into a CE emulsion sample for 30–60 min at both room temperature and $37^\circ C$. ^{19}F T1 measurements were made using inversion recovery spectroscopy with TR=10 s, and 10 inversion delays (TI) ranging from 3 ms to 5 s. To confirm that the linear response curve of CE NP on pO_2 was preserved in the blood, mouse blood (Bioreclamation, LLC, USA) containing 10% volume/volume ratio of CE NP was placed in a gas chamber to allow gas exchange through semi-permeable silicon tubing for 30 minutes. Blood was transferred to a sealed vial and the sample temperature was maintained at $37^\circ C$. For the linear fitting, the conversion of partial percentage of oxygen to mmHg was based on the assumption that

100% oxygen is equivalent to 713 mmHg (considering the vapor pressure at 37°C is 47 mmHg).

Noninvasive intravascular pO₂ measurement with BESR ¹⁹F imaging

Swiss Webster mice (n=5) were anesthetized with 1-2% isoflurane mixed with room air or O₂ followed by intravenous injection of CE NP (5 ml/kg) via the tail vein. The ¹⁹F signal from isoflurane was barely detectable by MRI in contrast to the strong ¹⁹F signal emanating from CE NP in the blood stream, which is likely due to low blood/tissue retention and the short T2* of isoflurane (27). ¹H gradient-echo cine images were acquired initially to position the slices to cover both left and right ventricles (LV and RV). Blood ¹⁹F T1 measurements were carried out under separate conditions of room air and then pure oxygen ventilation. The saddle coil and the surface coil were re-tuned as needed to the proper frequency of 201.5 MHz and 189.6 MHz for ¹H and ¹⁹F imaging, respectively. Parameters for ¹⁹F BESR T1 measurement were: TR, 2.5 s; TE, 2.2 ms; number of points along recovery curve, 8; number of averages, 16; in plane resolution, 1.5 mm × 0.75 mm; slice thickness, 2 mm. In order to optimize non-linear fitting for ¹⁹F T1 of less-oxygenated venous blood exhibiting T1 > 2 s, another set of BESR images acquired with identical imaging parameters but without pre-saturation pulses were used as fully recovered signal. The previously measured ¹⁹F R1 vs pO₂ calibration curve was used for quantifying intravascular pO₂ in vivo.

Data analysis and Statistics

The left and right ventricles and vessel lumen were manually segmented on ¹H and ¹⁹F images. ROI was defined as the region exhibiting > 3 SD higher ¹⁹F signal intensity, i.e. signal contributed by PFC NPs in the blood, as compared with that of surrounding tissues. The average intensity in each ROI was used for fitting data to a 3-parameter saturation recovery curve:

$$S(TS) = A - Be^{-TS/T_1} \quad [2]$$

All statistical analysis was performed using Origin software (OriginLab, USA). Metrics for precision and accuracy of T1 measurements with the BESR sequence were assessed based on ¹H image of the tubing phantom. The precision error was calculated as the root-mean-square of 5 individual measurement normalized by the mean value, and the accuracy error was considered to be the relative difference between the T1 mean value measured using a reference method and the BESR sequence. A two sample paired *t*-test was used for statistical analysis to test the difference between BESR measured ¹H T1 values and reference ¹H T1 values in vivo, and the difference of ¹⁹F MRI determined pO₂ between hyperoxic and normoxic conditions. A significance threshold of *p* < 0.05 was used for hypothesis testing.

Results

Mesurement of the homogeneity of pulse train saturation

The flip angle mapping of the transmit coil and the percentage residual magnetization intensity after different number of saturation pulses (1, 3, and 5) were measured experimentally and shown in Fig. 2. As the number of saturation pulses increased, spin nulling in the whole FOV became more uniform despite the inhomogeneity of the B1 profile. After five consecutive 90° pulses, the residual magnetization within the ROI where mice were placed for in vivo experiments (border marked by yellow dash line) was < 4%, substantially lower than the > 20% residual magnetization after a single saturation pulse.

Validation of BESR sequence determined T1

The ^1H T1 measured in the tube phantom and in arterial blood flow is summarized as follows: BESR sequence determined ^1H T1 of circulating water (2.96 ± 0.43 s) was comparable to that of stationary water measured with a traditional saturation recovery sequence (3.30 ± 0.17 s, N.S.). The precision and accuracy errors were 13.0% and 10.3%, respectively. The precision and accuracy errors provide quantitative estimates for the potential measurement bias and uncertainty, respectively. The in vivo measured ^1H T1 in arterial blood (on 100% O_2) in left and right carotid arteries and left ventricle were 1.62 ± 0.12 s, 1.59 ± 0.2 s, and 1.67 ± 0.14 s, respectively. The corresponding in vitro T1 of sampled arterial blood saturated with 100% O_2 was 1.78 ± 0.18 s (N.S. compared to all in vivo measurements).

In vitro calibration of ^{19}F R1 as a function of pO_2

In vitro measurements validated the linear dependence of ^{19}F R1 on pO_2 (Fig. 3). At 37 °C,

$$R1(s^{-1}) = (0.396 \pm 0.014) + (0.0024 \pm 0.0003) \times \text{pO}_2(\text{mmHg}). \quad [3]$$

Deoxy-Hb did not exhibit any observable paramagnetic relaxation enhancement effect on ^{19}F T1, because ^{19}F R1 vs pO_2 curve was identical in aqueous solution and mouse blood.

In vivo measurement of blood oxygenation

Figure 4 shows representative BESR images of a mouse heart under hyperoxia and normoxia. After spin saturation, blood in the left ventricle (LV) exhibited a faster recovery of signal intensity than that in the right ventricle (RV), which was further accelerated when the mouse was breathing pure O_2 (i.e. hyperoxia). The fitted ^{19}F R1 confirmed the faster relaxation of the more oxygenated blood. Based on the calibrated correlation between ^{19}F R1 and pO_2 (Eq. 3), ^{19}F MRI-measured LV blood pO_2 was 458 ± 133 mmHg under hyperoxia and 96 ± 21 mmHg under normoxia; significantly higher than RV blood pO_2 , which was 103 ± 21 mmHg under hyperoxia and 49 ± 15 mmHg under normoxia (Fig. 5, $p < 0.05$ for all comparisons).

Accuracy of pO_2 measurements at different SNR

Since low SNR is a common problem in ^{19}F MRI, the effect of SNR on ^{19}F BESR determined pO_2 was evaluated by mathematical simulation (Fig. 6). As expected, the accuracy of ^{19}F MRI measurements of pO_2 increased with SNR. However, the pO_2 measurement was less susceptible to noise (in the sense of absolute measurement uncertainty) at lower oxygen tension. When image SNR = 9, the measurement error of ^{19}F MRI determined pO_2 was <10 mmHg under hypoxic condition ($\text{pO}_2 < 50$ mmHg) and <40 mmHg under hyperoxic condition ($\text{pO}_2 > 500$ mmHg). Phantom studies with varying concentrations of PFC NPs (Fig. 6b) confirmed the simulation result. Higher imaging SNR substantially reduced the uncertainty and increased the precision of the ^{19}F R1 measurements.

Discussion

In the present work, we implemented a novel BESR sequence to quantify blood pO_2 based on ^{19}F R1 of circulating PFC NPs. Because ^{19}F R1 of PFC NPs is not affected by the paramagnetic effect of deoxy-Hb and manifests a linear association/release curve as a function of dissolved $[\text{O}_2]$, it represents a promising probe to quantify blood pO_2 . Our results demonstrate that the BESR sequence: 1) achieves a consistent T1 measurement for

pulsatile blood flow, 2) differentiates blood pO_2 in the LV and RV, and 3) depicts the response of ventricular blood oxygenation to hyperoxic challenge.

One concern regarding the use of any oxygen sensing probe in vivo is whether other molecules or ions will affect the chemical properties of the probe. In this study, we observed that pO_2 - ^{19}F R1 relationship remained unchanged for PFC NPs in salt solution and blood sample. This is consistent with our previous findings that ^{19}F R1 of PFC NPs is not affected by surrounding free paramagnetic ions (16,28) since their direct interactions are prevented by the lipid monolayer of PFC NPs, which is permeable to O_2 but not to macromolecules. The observed linear pO_2 response curve of PFC NP agreed with that observed in previous reports despite the difference in the fitted coefficient at different magnet field strength (12,29-30). The observed temperature dependence of ^{19}F R1 has also been previously reported (15).

The long circulating half life time of PFC NPs and high ^{19}F spin density are advantageous to use PFC NPs as a blood pool pO_2 probe. The mean half life of PFC NPs in the blood plasma is ~180 minutes in mouse and even longer in humans (31). Thus, the slow clearance of intravascular PFC NPs allows a relatively wide temporal window for quantitative pO_2 assessment with ^{19}F MRI. In addition, even though PFC has high affinity for O_2 , the modest administered dose is small enough such that the total amount of dissolved O_2 carried by PFC NPs is orders of magnitude less than that carried by plasma and red blood cells (dissolved plus oxy-hemoglobin) (32-34). Therefore, the blood-pool PFC NPs acted as a pO_2 sensor rather than a primary O_2 reservoir and the ^{19}F MRI measured pO_2 values reflects pO_2 in blood stream.

In major arteries (or ventricles), and veins, the velocity of blood flow is > 10 cm/s and 1 cm/s, respectively. For a typical imaging slice thickness (1-2 mm), the minimal time required for blood to flow through the slice is approximately 100-200 ms. By setting the time interval between two consecutive excitation pulses greater than 200 ms and selecting imaging slices perpendicular to the flow direction, the previously excited spins should not affect the subsequent image acquisitions. Thus, gradient echo imaging with 90° excitation pulses can achieve maximal SNR without partial saturation effects. On the other hand, because the blood flow is rapid in major blood vessels, during the recovery process of the T1 measurement the real “imaged” spins within the region of interest may originate from distant locations. This requires homogeneous spin pre-saturation over a large volume of the imaged subject. Consistent with previous studies that utilized pre-saturation pulse trains (33,35), our result suggests that a non-selective saturation pulse train with crusher gradients was robust for reducing both B_0 and B_1 inhomogeneity while achieving a satisfactory uniform spin saturation profile. By implementing multiple saturation recovery acquisitions in each TR, the BESR sequence achieved higher SNR than traditional saturation recovery or inversion recovery T1 measurement sequences.

The novelty of BESR resides in the utilization of a homogenous RF pre-saturation and a train of ECG gated excitation/readouts in a single TR for fast T1 quantification. Due to the refreshing of intravascular PFC NP in the imaging slice (thickness = 2 mm) between adjacent heart beats, the acquired blood-pool ^{19}F signal by each readout directly represents the ^{19}F T1 recovery at corresponding delay times after saturation. In contrast, the conventional saturation pulse sequence only acquires one echo after each RF saturation, and the different T1 recovery weighting is achieved by varying TR (36). Thus, the BESR sequence provides a faster imaging method for quantifying blood ^{19}F T1 by taking advantage of the time-of-flight effect of blood-flow in conjunction with intravascular PFC NP contrast agent. Although multiple 90° pulses were used in this study, other pulse shapes such as adiabatic or composite pulses also could be employed as long as homogeneous

whole-body saturation is achieved (37). Similarly, fast imaging techniques like EPI could replace the single echo to further accelerate image acquisition (36).

The BESR sequence is optimized for ^{19}F MRI because: (a) the PFC NP is an intravascular agent; (b) there is no ^{19}F background in soft-tissue; (c) O_2 dissolving and release from PFC NP (~250 nm) operates through free diffusion that occurs in milliseconds (38). In capillaries, the measured pO_2 may directly reflect tissue pO_2 if the O_2 exchange between tissue and microvasculature is rapid (39). In ventricles and major blood vessel that experiencing no blood-tissue O_2 exchange, the measured pO_2 would represent venous or arterial blood pO_2 . Finally, although the BESR sequence could be applied to quantify ^1H T1 in blood pool, the small correlation coefficient between ^1H R1 and pO_2 may be problematic for accurate pO_2 determination. Additionally, the O_2 release response curve of hemoglobin is non-linear, therefore the accuracy of ^1H MRI measured blood pO_2 , as well as its relationship with tissue pO_2 , remains to be defined.

The proposed ^{19}F imaging methodology has direct translational potential. The technique might be readily adapted to evaluate arteriovenous pO_2 difference for selected organs, e.g. renal arteries and veins for kidney, carotid arteries and jugular veins for brain, aorta and coronary sinus for heart, among others. Further integration of ^{19}F MRI determined A-V pO_2 with phase-contrast ^1H or ^{19}F MRI determined blood flow velocity may enable assessment of oxygen consumption in individual organs as a critical pathological marker for evaluating injury at a cellular level.

Based on the accuracy profile of ^{19}F -determined pO_2 , imaging noise significantly affects the measurement uncertainty of pO_2 through non-linear T1 fitting. Therefore, the acquisition of ^{19}F images with sufficient SNR is crucial to accurately estimate intravascular pO_2 , which also was reported previously by Barker et al (14). In the present study, all images exhibited $\text{SNR} > 7$, and according to our simulation (Fig. 6a), pO_2 estimates from these images could achieve a measurement uncertainty as low as 10 mmHg under normoxia and 30 mmHg under hyperoxia depending on real pO_2 .

Several limitations of the current pO_2 measurement paradigm should be mentioned. Even though the blood flow in major blood vessels is fast enough to create “time-of-flight” phenomenon, the velocity profile of blood flow is laminar, where the velocity close to the blood vessels walls is virtually zero. This portion of the blood may contribute to ^{19}F signal resulting in reduced accuracy of pO_2 measurement in such regions (22). In the cardiac ventricles and major vessels, non laminar blood flow (e.g., turbulence) also may cause errors in T1 estimation due to spin de-correlation, which is not unexpected and can be visualized on ^1H images for proper data interpretation (40). Because the ejection fraction of normal ventricle is around 60%, after two heart beats around 16% residual blood still remains in the same ventricle. The residual blood may suffer from partial saturation from previous excitation and cause errors in T1 measurement. The systematic error (about 6% of T1 measured in phantom experiments) caused by these factors may be corrected by flow dynamical modeling in the future. The relative error of ^{19}F BESR MRI determined blood pO_2 , (i.e. 50 mmHg) in well oxygenated arterial blood or in venous blood (10 mmHg), could limit the application of this technique for measurement of tumor oxygenation, but there are other uses that offer insights into myriad pathophysiologies. Finally, for human imaging, where sampling once per cardiac cycle is too slow because of lower heart rate, the sequence should be modified to image more than once per cardiac cycle.

Acknowledgments

We thank Ralph W. Fuhrhop and Angana Senpan for preparing PFC NP. We also thank Mark S. Conradi for helpful discussion. We acknowledge the financial support from NIH Grants U54 CA119342 and R01 HL073646.

References

1. Rudolph, AM. Congenital diseases of the heart : clinical-physiological considerations. Vol. viii. Armonk, NY: Futura Pub. Co.; 2001. p. 808
2. Terry PB, White RI, Barth KH, Kaufman SL, Mitchell SE. Pulmonary arteriovenous-malformations - physiologic observations and results of therapeutic balloon embolization. *N Engl J Med*. 1983; 308(20):1197–1200. [PubMed: 6405268]
3. Fedele FA, Gewirtz H, Capone RJ, Sharaf B, Most AS. Metabolic response to prolonged reduction of myocardial blood flow distal to a severe coronary artery stenosis. *Circulation*. 1988; 78(3):729–735. [PubMed: 3409507]
4. Grossman, W. Cardiac catheterization and angiography. Vol. xiii. Philadelphia: Lea & Febiger; 1986. p. 562
5. Silvennoinen MJ, Clingman CS, Golay X, Kauppinen RA, van Zijl PC. Comparison of the dependence of blood R2 and R2* on oxygen saturation at 1.5 and 4.7 Tesla. *Magn Reson Med*. 2003; 49(1):47–60. [PubMed: 12509819]
6. Fujima N, Kudo K, Terae S, Hida K, Ishizaka K, Zaitzu Y, Asano T, Yoshida D, Tha KK, Haacke EM, Sasaki M, Shirato H. Spinal arteriovenous malformation: evaluation of change in venous oxygenation with susceptibility-weighted MR imaging after treatment. *Radiology*. 2010; 254(3): 891–899. [PubMed: 20177100]
7. Kershaw LE, Naish JH, McGrath DM, Waterton JC, Parker GJ. Measurement of arterial plasma oxygenation in dynamic oxygen-enhanced MRI. *Magn Reson Med*. 2010; 64(6):1838–1842. [PubMed: 20677232]
8. Nield LE, Qi X, Yoo SJ, Valsangiacomo ER, Hornberger LK, Wright GA. MRI-based blood oxygen saturation measurements in infants and children with congenital heart disease. *Pediatr Radiol*. 2002; 32(7):518–522. [PubMed: 12107586]
9. Mason RP, Jeffrey FM, Malloy CR, Babcock EE, Antich PP. A noninvasive assessment of myocardial oxygen tension: 19F NMR spectroscopy of sequestered perfluorocarbon emulsion. *Magn Reson Med*. 1992; 27(2):310–317. [PubMed: 1334204]
10. Shukla HP, Mason RP, Bansal N, Antich PP. Regional myocardial oxygen tension: 19F MRI of sequestered perfluorocarbon. *Magn Reson Med*. 1996; 35(6):827–833. [PubMed: 8744009]
11. Fishman JE, Joseph PM, Floyd TF, Mukherji B, Sloviter HA. Oxygen-sensitive 19F NMR imaging of the vascular system in vivo. *Magn Reson Imaging*. 1987; 5(4):279–285. [PubMed: 3657400]
12. Duong TQ, Kim SG. In vivo MR measurements of regional arterial and venous blood volume fractions in intact rat brain. *Magn Reson Med*. 2000; 43(3):393–402. [PubMed: 10725882]
13. Eidelberg D, Johnson G, Barnes D, Tofts PS, Delpy D, Plummer D, McDonald WI. 19F NMR imaging of blood oxygenation in the brain. *Magn Reson Med*. 1988; 6(3):344–352. [PubMed: 3362067]
14. Barker BR, Mason RP, Bansal N, Peshock RM. Oxygen tension mapping with F-19 echo-planar MR imaging of sequestered perfluorocarbon. *J Magn Reson Imaging*. 1994; 4(4):595–602. [PubMed: 7949687]
15. Liu S, Shah SJ, Wilmes LJ, Feiner J, Kodibagkar VD, Wendland MF, Mason RP, Hylton N, Hopf HW, Rollins MD. Quantitative tissue oxygen measurement in multiple organs using (19) F MRI in a rat model. *Magn Reson Med*. 2011; 66(6):1722–30. [PubMed: 21688315]
16. Hu L, Zhang L, Chen J, Lanza GM, Wickline SA. Diffusional mechanisms augment the fluorine MR relaxation in paramagnetic perfluorocarbon nanoparticles that provides a “relaxation switch” for detecting cellular endosomal activation. *J Magn Reson Imaging*. 2011; 34(3):653–661. [PubMed: 21761488]
17. Cohn CS, Cushing MM. Oxygen therapeutics: perfluorocarbons and blood substitute safety. *Crit Care Clin*. 2009; 25(2):399–414. [PubMed: 19341916]
18. Wickline, SA.; Mason, RP.; Caruthers, SD.; Chen, J.; Winter, PM.; Hughes, MS.; Lanza, GM. Fluorocarbon agents for multimodal molecular imaging and targeted therapeutics. In: Weissleder, R.; Ross, BD.; Rehemtulla, A.; Gambhir, SS., editors. *Molecular imaging: Principles and practice*. Peoples Medical Publishing House; USA: 2010. p. 42-573.

19. Wu WC, Jain V, Li C, Giannetta M, Hurt H, Wehrli FW, Wang DJ. In vivo venous blood T1 measurement using inversion recovery true-FISP in children and adults. *Magn Reson Med*. 2010; 64(4):1140–1147. [PubMed: 20564586]
20. Thomas DL, Lythgoe MF, Gadian DG, Ordidge RJ. In vivo measurement of the longitudinal relaxation time of arterial blood (T1a) in the mouse using a pulsed arterial spin labeling approach. *Magn Reson Med*. 2006; 55(4):943–947. [PubMed: 16526019]
21. Zheng J, Venkatesan R, Haacke EM, Cavagna FM, Finn PJ, Li D. Accuracy of T1 measurements at high temporal resolution: feasibility of dynamic measurement of blood T1 after contrast administration. *J Magn Reson Imaging*. 1999; 10(4):576–581. [PubMed: 10508325]
22. Guo JY, Kim SE, Parker DL, Jeong EK, Zhang L, Roemer RB. Improved accuracy and consistency in T1 measurement of flowing blood by using inversion recovery GE-EPI. *Med Phys*. 2005; 32(4):1083–1093. [PubMed: 15895593]
23. Dumoulin CL, Buonocore MH, Opsahl LR, Katzberg RW, Darrow RD, Morris TW, Batey C. Noninvasive measurement of renal hemodynamic functions using gadolinium enhanced magnetic resonance imaging. *Magn Reson Med*. 1994; 32(3):370–378. [PubMed: 7984069]
24. Cunningham CH, Pauly JM, Nayak KS. Saturated double-angle method for rapid B1+ mapping. *Magn Reson Med*. 2006; 55(6):1326–1333. [PubMed: 16683260]
25. Bernstein, MA.; King, KF.; Zhou, ZJ. Handbook of MRI pulse sequences. Vol. xxii. Amsterdam; Boston: Academic Press; 2004. p. 1017
26. Roth DM, Swaney JS, Dalton ND, Gilpin EA, Ross J Jr. Impact of anesthesia on cardiac function during echocardiography in mice. *Am J Physiol Heart Circ Physiol*. 2002; 282(6):H2134–2140. [PubMed: 12003821]
27. Chen M, Olsen JJ, Stolk JA, Schweizer MP, Sha M, Ueda I. An in vivo ¹⁹F NMR study of isoflurane elimination as a function of age in rat brain. *NMR Biomed*. 1992; 5(3):121–126. [PubMed: 1642968]
28. Neubauer AM, Myerson J, Caruthers SD, Hockett FD, Winter PM, Chen JJ, Gaffney PJ, Robertson JD, Lanza GM, Wickline SA. Gadolinium-Modulated F-19 Signals From Perfluorocarbon Nanoparticles as a New Strategy for Molecular Imaging. *Magnetic Resonance in Medicine*. 2008; 60(5):1066–1072. [PubMed: 18956457]
29. Fan X, River JN, Muresan AS, Popescu C, Zamora M, Culp RM, Karczmar GS. MRI of perfluorocarbon emulsion kinetics in rodent mammary tumours. *Phys Med Biol*. 2006; 51(2):211–220. [PubMed: 16394334]
30. Kadayakkara DK, Janjic JM, Pusateri LK, Young WB, Ahrens ET. In vivo observation of intracellular oximetry in perfluorocarbon-labeled glioma cells and chemotherapeutic response in the CNS using fluorine-19 MRI. *Magn Reson Med*. 2010; 64(5):1252–1259. [PubMed: 20860007]
31. Spahn DR. Blood substitutes. Artificial oxygen carriers: perfluorocarbon emulsions. *Crit Care*. 1999; 3(5):R93–97. [PubMed: 11094488]
32. Thomas SR, Pratt RG, Millard RW, Samarasinghe RC, Shiferaw Y, Clark LC Jr, Hoffmann RE. Evaluation of the influence of the aqueous phase bioconstituent environment on the F-19 T1 of perfluorocarbon blood substitute emulsions. *J Magn Reson Imaging*. 1994; 4(4):631–635. [PubMed: 7949694]
33. Mason RP, Shukla H, Antich PP. In vivo oxygen tension and temperature: simultaneous determination using ¹⁹F NMR spectroscopy of perfluorocarbon. *Magn Reson Med*. 1993; 29(3):296–302. [PubMed: 8450738]
34. Lai C-S, Stair SJ, Mizioro H, Hyde JS. Effect of oxygen and the lipid spin label TEMPO-laurate on fluorine-19 and proton relaxation rates of the perfluorochemical blood substitute, FC-43 emulsion. *Journal of Magnetic Resonance* (1969). 1984; 57(3):447–452.
35. Evelhoch JL, Ackerman JH. NMR T1 measurements in inhomogeneous B1 with surface coils. *Journal of Magnetic Resonance* (1969). 1983; 53(1):52–64.
36. Le D, Mason RP, Hunjan S, Constantinescu A, Barker BR, Antich PP. Regional tumor oxygen dynamics: ¹⁹F PBSR EPI of hexafluorobenzene. *Magn Reson Imaging*. 1997; 15(8):971–981. [PubMed: 9322216]
37. Tannus A, Garwood M. Adiabatic pulses. *NMR Biomed*. 1997; 10(8):423–434. [PubMed: 9542739]

38. O'Brien RN, Langlais AJ, Seufert WD. Diffusion coefficients of respiratory gases in a perfluorocarbon liquid. *Science*. 1982; 217(4555):153–155. [PubMed: 6806902]
39. Diepart C, Magat J, Jordan BF, Gallez B. In vivo mapping of tumor oxygen consumption using (19) F MRI relaxometry. *NMR Biomed*. 2011; 24(5):458–463. [PubMed: 20891023]
40. Ehman RL, Felmlee JP. Flow artifact reduction in MRI: a review of the roles of gradient moment nulling and spatial presaturation. *Magn Reson Med*. 1990; 14(2):293–307. [PubMed: 2345509]

Figure 1.

Illustration of the Blood flow-Enhanced-Saturation-Recovery (BESR) sequence. Spin preparation is achieved with multiple non-selective saturation pulses; image acquisition uses a gradient echo approach with cardiac triggering; saturation pre-pulse train and gradient echo imaging are both triggered by ECG. Each image acquisition is acquired at the same trigger delay time t but corresponds to different saturation delay time TS .

Figure 2. (a, b) Flip angle mapping of a water phantom in axial and coronal plane. (c, d) Measured residual magnetization after different number of saturation pulses (NSP = 1, 3, 5) in axial and coronal plane. The yellow dashed lines represent the border of the ROI for in vivo study. The residual magnetization inside the ROI is presented as mean $\pm$ standard deviation.

Figure 3.

Calibrated linear relationship between $^{19}\text{F} R_1$ and pO_2 at room temperature and 37° . The calibration curve at 37° is used for the in vivo study. Error bars represent standard deviation.

Figure 4.

(a) A short-axis ^1H image shows the left and right ventricles. (b) ^{19}F BESR images of the same mouse heart under hyperoxia and normoxia. (c-d) Fitted ^{19}F saturation recovery curve for LV and RV blood under normoxia and hyperoxia. Error bars represent standard deviation.

Figure 5. Measured blood pO₂ based on measured ¹⁹F T1 of PFC NPs showed pO₂ difference between LV and RV, and between normoxic and hyperoxic conditions for each ventricle, *, p<0.05.

Figure 6.

(a) Simulation result shows measurement accuracy improves as SNR increases. pO_2 measurement uncertainty is higher under hyperoxic condition. (b) Measured pO_2 for three PFC NPs samples with different NP concentration bubbled with N_2 , Air and O_2 , respectively. The mean value of measured pO_2 is independent on PFC NPs concentration but measurement uncertainty (error bars) is suppressed by increased SNR.