

Fluorine Cardiovascular Magnetic Resonance Angiography *In Vivo* at 1.5 T with Perfluorocarbon Nanoparticle Contrast Agents

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ABSTRACT

While the current gold standard for coronary imaging is X-ray angiography, evidence is accumulating that it may not be the most sensitive technique for detecting unstable plaque. Other imaging modalities, such as cardiovascular magnetic resonance (CMR), can be used for plaque characterization, but suffer from long scan and reconstruction times for determining regions of stenosis. We have developed an intravascular fluorinated contrast agent that can be used for angiography with cardiovascular magnetic resonance at clinical field strengths (1.5 T). This liquid perfluorocarbon nanoparticle contains a high concentration of fluorine atoms that can be used to generate contrast on ¹⁹F MR images without any competing background signal from surrounding tissues. By using a perfluorocarbon with 20 equivalent fluorine molecules, custom-built RF coils, a modified clinical scanner, and an efficient steady-state free precession sequence, we demonstrate the use of this agent for angiography of small vessels *in vitro*, *ex vivo*, and *in vivo*. The surprisingly high signal generated with very short scan times and low doses of perfluorocarbon indicates that this technique may be useful in clinical settings when coupled with advanced imaging strategies.

INTRODUCTION

The standard technique for diagnosis and evaluation of coronary artery stenosis is cardiovascular catheterization. Over one million diagnostic catheterizations are performed each year in the United States, generally after a patient has presented with symptoms of angina or myocardial infarction. However, cardiac catheterization is an invasive technique that has the potential to incur complications, and the exposure to ionizing radiation may produce unwanted long-term side effects in both patients and operators. Furthermore, lumen area has only been shown to decrease after approximately 40% of the vessel becomes filled

with plaque, due to positive remodeling (1). Often these plaques, which would not be identified with traditional cardiac catheterization, exhibit characteristics that make them vulnerable to rupture and may instigate a major ischemic event (2, 3). Future progress in reducing deaths from atherosclerosis may require the use of additional techniques that may provide more information about a particular plaque (4).

Alternative imaging techniques, such as cardiovascular magnetic resonance (CMR), intravascular ultrasound (IVUS), cardiac computed tomography (CT), optical coherence tomography, angiography, and thermography are being evaluated for their ability not only to diagnose vessel stenosis, but also to characterize plaque composition (5, 6). Of these modalities, CMR is unique in that it is noninvasive and does not require exposure to ionizing radiation. The most common method for imaging the coronary arteries with CMR employs a motion gated, 3D acquisition from which the imaging plane containing the vessel can be reconstructed (7, 8). This technique generates high-resolution images of the coronary arteries. Sites of vessel narrowing and irregularities can then be examined in more detail using higher resolution scans to determine plaque composition (9). However, acquisition and reconstruction time can be prohibitive when compared to cardiac catheterization, and compounding variables

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such as vessel tortuosity and changes in blood flow further increase the probability of false positives. The development of a method for rapid imaging of the vessel lumen with CMR would be advantageous for delineating stenotic regions, while also permitting the acquisition of high resolution functional exams and plaque characterization.

In this work, we demonstrate a technique for CMR angiography that uses a fluorine-based contrast agent and ^{19}F MR. The fluorine atom nucleus contains one unpaired neutron, and thus exhibits a net spin, which makes it visible with magnetic resonance techniques. In addition, its gyromagnetic ratio (40.08 MHz/T) is only slightly lower than that of ^1H nuclei (42.58 MHz/T), so that its relative sensitivity, which is proportional to the gyromagnetic ratio to the third power, is 83% when compared to ^1H . These properties encourage the exploration of fluorine contrast agents for CMR studies, especially since biological tissues contain little to no endogenous fluorine that can be detected by CMR/MRS. Unlike standard ^1H MR imaging, fluorine contrast angiography has the advantage that any signal that is generated can be definitively attributed to the contrast agent itself, making identification of the lumen and other structures unique. The use of ^{19}F CMR has been demonstrated in the past for a variety of applications (10–13), and more recent work has shown that it can be used to enhance target-specific imaging (14–16).

Our goal in this work is to demonstrate the concept of fluorine imaging for CMR angiography in conjunction with the advanced tools of present-day imaging technology to improve signal-to-noise and speed of data acquisition. We employ several techniques to increase the spatial and temporal resolution, while reducing the required dose of contrast agent to the subject. These include the use of a cyclic perfluorocarbon molecule (perfluoro-15-crown-5-ether) that comprises 20 chemically identical fluorine atoms, the use of custom-built RF coils for increased sensitivity, and the use of a steady-state imaging technique that vastly accelerates data acquisition while maintaining the fluorine signal level. These techniques were employed in selected situations to characterize the capability of our method for ^{19}F CMR angiography of small vessels *in vivo*. The ultimate goal of this work is to illustrate the potential of ^{19}F CMR to serve as a tool for noninvasive angiography under clinical imaging conditions.

METHODS

Nanoparticle formulation

Nanoparticle emulsions were formulated as described previously (17, 18). Briefly, they were composed of 20% (v/v) of perfluoro-15-crown-5 ether ($\text{C}_{10}\text{F}_{20}\text{O}_5$; Exflur Research Corp., Round Rock, Texas, USA), 1.5% (w/v) of a surfactant co-mixture, and 1.7% (w/v) glycerin, with water comprising the balance. The surfactant co-mixture was comprised of lipophilic gadolinium-diethylene-triamine-pentaacetic acid-bis-oleate (Gd-DTPA-BOA; Gateway Chemical Technologies, St. Louis, Missouri, USA), lecithin (Avanti Polar Lipids, Inc., Alabaster, Alabama, USA), and dipalmitoylphosphatidylethanolamine (Avanti Polar Lipids, Inc.) in a molar

ratio of 30:68:2. Particle sizes were determined at 25°C with a laser light scattering submicron particle sizer (Malvern Zetasizer Instruments, Malvern, Worcestershire, UK). Perfluoro-15-crown-5 ether (hereafter referred to as CE) is a cyclic perfluorocarbon with 20 equivalent fluorine atoms per molecule. The ^{19}F relaxation parameters were measured at 1.5 T in triplicate using inversion recovery spectroscopy with 10 inversion times for T_1 , and multi-echo spin echo spectroscopy with 10 different echo times for T_2 . Values were measured after the samples had time to equilibrate to room temperature (22°C) and ambient oxygen levels using a volume coil tuned to 40.1 MHz. These data were analyzed using NMR Utility Transform Software, NUTS (Acorn NMR, Livermore, California, USA).

MR system and coil development

All imaging was performed on a 1.5 T clinical MR system (NT Intera, Philips Medical Systems, Andover, Massachusetts, USA) with peak gradients of 30 mT/m (150 mT/m/ms) and outfitted with a secondary radiofrequency (RF) transmit/receive system tuned for ^{19}F . All RF coils used for ^{19}F imaging were built in-house and used for both transmission and receive of the CMR signal. A single element, linear volume coil was fabricated using a saddle coil design to be 13.5 cm diameter and 14.5 cm long using copper foil formed onto a plexiglass frame. High-voltage variable capacitors made of Teflon for MR compatibility (Johanson, Boonton, New Jersey, USA and Voltronics, Denville, New Jersey, USA) were used for tuning and matching to different loads, and a custom-built balun network was added for improved isolation. To increase the sensitivity for *in vivo* imaging, a 7 cm single loop surface coil was created by chemical etching of copper-clad glass epoxy. Variable tuning and matching capacitors were used to accommodate different loads, and splitting of the matching capacitance provided adequate isolation. The unloaded Q of these coils as measured at 60.18 MHz with a network analyzer (Agilent Technologies) was 152 and 174 for the saddle and surface coil, respectively.

Phantom imaging

Phantom imaging was used for the initial testing of this system for fluorine angiography. Flexible plastic extension tubing (Baxter Healthcare Corp, Deerfield, Illinois, USA; ~1.9 mm diameter) was formed into a loop and placed inside of the saddle coil (described above) between saline IV bags to minimize susceptibility artifact. Undiluted crown ether (CE) nanoparticles were slowly injected into the tubing (~1.8 mL/min flow rate), and the ^{19}F signal was imaged using a dynamic steady-state free precession imaging sequence (balanced FFE, or bFFE) with the following parameters: 4 ms TR, 1.4 ms TE, 320 mm FOV, $2.5 \times 2.0 \times 73$ mm reconstructed resolution, 4 signal averages, 90° flip angle, 1.3 seconds per image acquisition. ^1H multislice images were also acquired for co-localization of the ^{19}F signal using the built-in quadrature body coil (turbo spin echo sequence with turbo factor of 22, 5 slices, 1518 ms TR, 150 ms TE, 320 mm FOV, $1.25 \times 1.01 \times 6$ mm resolution, 6 signal averages).

Ex vivo angiography

For further characterization of this technique using a controlled sample, we chose to use an isolated heart. The coronary vessels, which bend and branch, would provide a measure of how sensitive this technique could be for identifying vasculature under ideal conditions (no flow, no movement, high local concentration of nanoparticles). The heart from a heparanized pig was removed, the coronaries were flushed with saline, and the entire sample was placed in formalin until imaging time (approximately 24 hours). A 4F Fogarty catheter (Edwards Lifesciences, Irvine, California, USA) with the balloons removed to avoid artifacts on the image was placed into the left anterior descending (LAD) coronary artery and affixed using sutures to prevent dislodging and backflow of fluid. Undiluted CE nanoparticles were slowly injected into the catheter, during which dynamic projection ^{19}F images were acquired using the saddle coil (bFFE sequence, 4 ms TR, 1.5 ms TE, 260 mm FOV, $1.4 \times 2.0 \times 70$ mm reconstructed resolution, 10 signal averages, 90° flip angle, 1.8 seconds per image acquisition). Multislice ^1H images were also acquired using the built-in quadrature body coil (3D FFE sequence, 30 slices, 320 mm FOV, 6.2 ms TR, 2.0 ms TE, $1.25 \times 1.24 \times 1.5$ mm reconstructed resolution, 10 signal averages, 90° flip angle). All animal procedures and experimental protocols used in this study were approved by the Washington University animal care committee.

Fluorine angiography of rabbit carotids

The next set of experiments is designed to evaluate how well this technique translates to in vivo imaging conditions. We tested this in four different rabbits (Harlan, Indianapolis, IN) two of which were catheterized to allow direct injection of a high concentration of particles. Male New Zealand white rabbits ($n = 2$) were anesthetized using an intramuscular injection of ketamine (35 mg/kg) and xylazine (7 mg/kg) followed by maintenance using IV delivery of a ketamine and xylazine (2 mg/kg/min and 1 mg/kg/min respectively) mixture. Rabbits were intubated and maintained on 2 L/min 100% O_2 for the duration of the exam. For first pass imaging, the rabbits were catheterized using a femoral artery cutdown technique under sterile conditions. A 4F Fogarty catheter (Edwards Lifesciences) was then advanced to the left carotid artery under fluoroscopy guidance. The animals were subsequently positioned in the MR scanner for imaging, and ^1H surveys and time-of-flight angiography scans of the neck region were acquired using the quadrature body coil for transmission and a 4 cm surface coil for receive (multiple 2D inflow FFE sequence, 160 mm FOV, 2.8 ms TE, 6.8 ms TR, 4 signal averages, 40 slices, $0.31 \times 0.31 \times 4$ mm reconstructed resolution, 60° flip angle, 2.3 min scan time). CE nanoparticles were injected (1–2 mL) into the vessel, during which ^{19}F dynamic projection images were acquired with a 7 cm surface coil (bFFE sequence, 260 mm FOV, 1.7 ms TE, 3.5 ms TR, 512 signal averages, $2.03 \times 2.03 \times 50$ mm reconstructed resolution, 90° flip angle, ~ 2 min total scan time).

In two additional rabbits, steady state intravascular fluorine concentrations were produced for angiographic imaging by ad-

ministering 2.5–3 mL/kg of crown ether nanoparticles in 0.5 mL/kg increments through the ear vein. This provided a mechanism for determining the lowest dose that provided a high enough signal to generate an image of the carotid arteries. Subsequent to each injection, ^{19}F projection images of the vessels in the neck were acquired (bFFE sequence, 260 mm FOV, 1.4 ms TE, 4 ms TR, 512 signal averages, $2.03 \times 2.03 \times 20$ mm reconstructed resolution, 60° flip angle, ~ 2 min total scan time). An intravenous blood sample (1 mL) was drawn from the contralateral ear at baseline (prior to injection) and after each image acquisition and analyzed for gadolinium content (see below). Total imaging time was approximately 2 hours. After completion of the study, all rabbits were euthanized with a potassium chloride injection (Euthosol, Delmarva Laboratories, Midlothian, Virginia, USA).

Analysis of blood samples for gadolinium content

To relate the ^{19}F signal intensity to concentration of fluorine, blood samples from each rabbit used for noninvasive angiography (i.e., no catheterization) were analyzed for gadolinium content using both relaxation time measurements and neutron activation analysis. The relationship between the gadolinium and fluorine content is determined by the molar ratio used during formulation. A benchtop spectrometer (MiniSpec, Bruker Optics, Billerica, Massachusetts, USA) at 0.47 T was used for relaxation time measurements. A calibration curve was obtained by doping blood from an untreated rabbit with known volumes of the crown ether, gadolinium-containing, nanoparticles. Six amounts, ranging from 0 to 20 μL of emulsion, were added to 0.5 mL of blood, producing fluorine concentrations of 0 to 0.49 M. An inversion recovery pulse sequence was used with 10 inversion delay times that varied according to the concentration of gadolinium present. A minimum of three T_1 measurements was averaged for each sample, and measurements were made at 40°C . Four of the six calibration samples were also prepared for neutron activation analysis for absolute quantification of the gadolinium content at the Research Reactor facility at the University of Missouri (MURR) (19, 20). Blood samples from the rabbits injected with nanoparticles were analyzed in a similar manner with both relaxation measurements and neutron activation for total gadolinium content. The relaxivity determination using the calibration samples allowed calculation of the absolute concentration of gadolinium in the blood samples, and neutron activation was used to verify this measurement.

RESULTS

The undiluted nanoparticles, as formulated with 20 vol% perfluorocarbon, contain 12.14 M fluorine atoms (or alternatively, 0.61 M perfluoro-15-crown-5 ether) and approximately 40,000 gadolinium atoms per particle. The nominal particle diameter was measured to be 185 nm. The longitudinal relaxivity of the particles was $12.3 \text{ s}^{-1} \text{ mM}^{-1}$ in rabbit blood at 40°C and 0.47 T, when expressed in terms of the concentration of gadolinium chelates (regression line: $R_1 (1/\text{s}) = 12.3 \cdot [\text{Gd}] (\text{mM})$

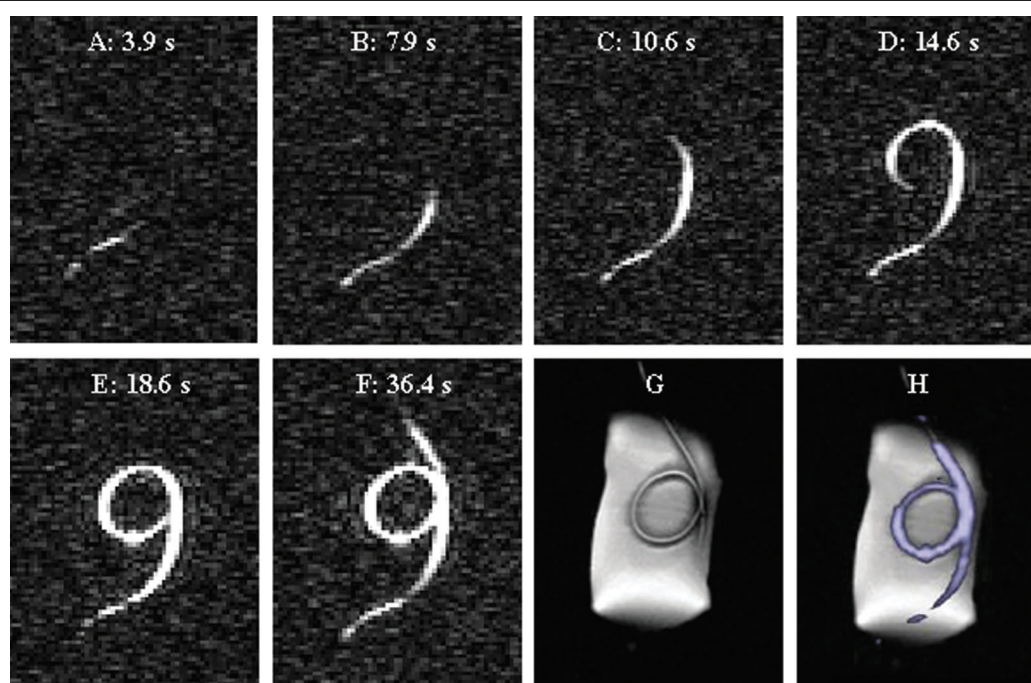


Figure 1. (A–F) Selected images from a series of dynamic ^{19}F images of undiluted crown ether nanoparticles flowing through plastic tubing. The time of acquisition after the injection started is labeled on the images in seconds. **G**, A ^1H single slice image of the tubing lying on top of a saline IV bag. **H**, False color overlay of image **F** onto image **G** showing the colocalization of the ^{19}F signal with the tubing.

+ 0.90; $r^2 > 0.99$), which is fairly consistent with the same measurements conducted in saline (20). The ^{19}F relaxation parameters measured at 1.5 T at room temperature and ambient oxygenation levels were 226.3 ± 3.3 and 64.4 ± 0.86 ms for T_1 and T_2 , respectively.

The initial phantom experiments conducted demonstrate the high level of signal that can be obtained using a clinical scanner and rapid bFFE imaging sequence (Fig. 1, A–F). Only selected images from the series are shown to demonstrate the movement of the particles through the tubing. This acquisition resulted in a signal-to-noise ratio of approximately 14, which is equivalent to the contrast-to-noise ratio because of the lack of competing background signal. Note, from **G** and **F** in Fig. 1, that the fluorine signal overlays precisely with the tubing in the ^1H image. In this example, the diameter of the tubing is consistent (1.9 mm) and the injection of undiluted nanoparticles provides the highest signal obtainable.

To demonstrate this technique with a physiologically relevant sample, we chose to use a pig heart. The left anterior descending (LAD) coronary artery manifests $\sim 2\text{--}3$ mm diameter lumen at its origin, but then branches into vessels much smaller than the tubing used in the phantom experiment. This would provide an estimation of the smallest vessels that can be visualized with the nanoparticles under conditions of no bulk flow, no anatomical motion, and no dilution. The LAD was visualized clearly with this technique, with the signal-to-noise ratio reaching 32 (Fig. 2, A–F). Some of the larger proximal branches were also visualized well, and smaller vessels were represented by a “blush” of ^{19}F signal, which increased over time to a signal-to-noise ratio of

10.7. These promising results provided motivation for further *in vivo* experimentation.

In vivo imaging of rabbit carotid arteries was more challenging due to dilution of the particles by the blood, anatomical motion (i.e., respiratory and cardiac), coil requirements, and the necessary temporal resolution. For added sensitivity, we utilized a surface coil tuned to 60.1 MHz for transmission and reception of the signal instead of a volume coil. The first set of images was acquired by placing the catheter in the carotid artery to permit delivery of high local concentrations of nanoparticles. Both the carotid arteries and jugular veins were readily detected in the images during nanoparticle injection (Fig. 3, B–E), despite the fact that the amount injected was not large enough to bring the average blood concentration high enough for visualization (Fig. 3, A). The ^{19}F signal is also demonstrated to coincide with the signal from the neck vasculature on a coronal maximum intensity projection (MIP) of a ^1H time of flight angiography scan (Fig. 3, F). As expected, dilution in the blood is a significant factor that degrades the available signal when compared to the phantom and *ex vivo* studies.

As a final step in evaluating this method for ^{19}F angiography, we injected nanoparticles systemically to determine the minimum dosage required for visualization of the neck vasculature. Each 0.5 mL/kg injection increased the systemic concentration of ^{19}F in the rabbit’s blood in a predictable manner (Fig. 4), due in part to the intravascular retention and long circulating half-life of these particles (21). For the first rabbit, a minimum of 3 doses (1.5 mL/kg) was required to detect the ^{19}F signal (Fig. 5, A). Each subsequent dose increased the signal, and generated

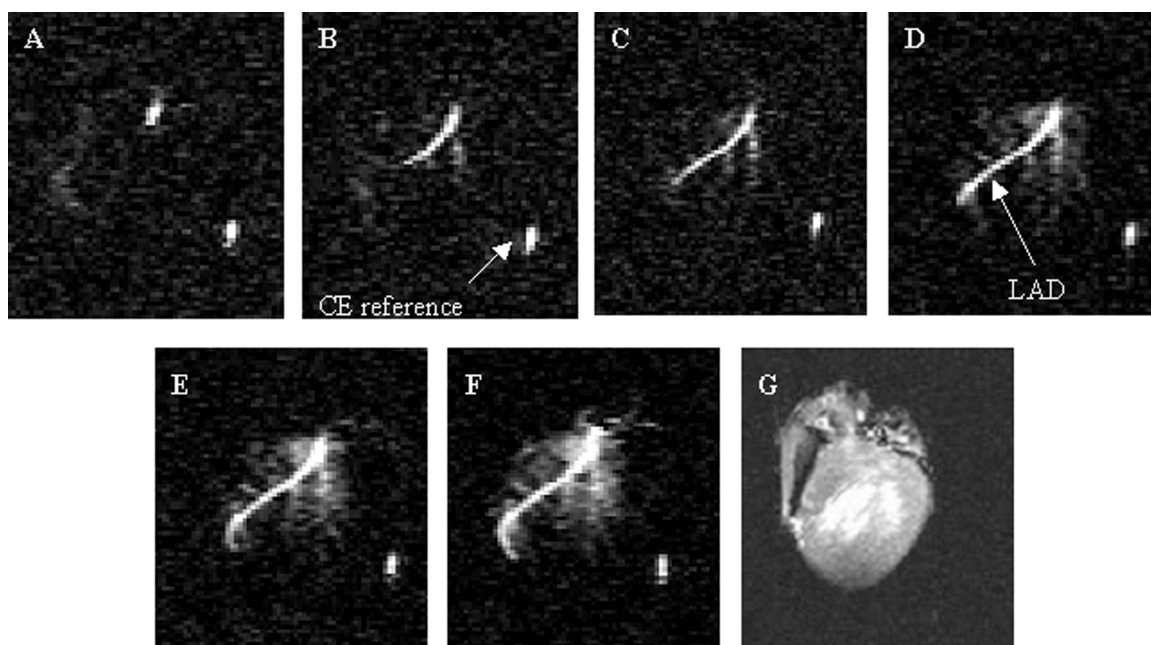


Figure 2. (A–F) Dynamic ^{19}F images of undiluted crown ether (CE) nanoparticles injected into the left anterior descending (LAD) coronary artery of an excised pig heart. G, ^1H single slice image of the same heart to demonstrate the underlying anatomy.

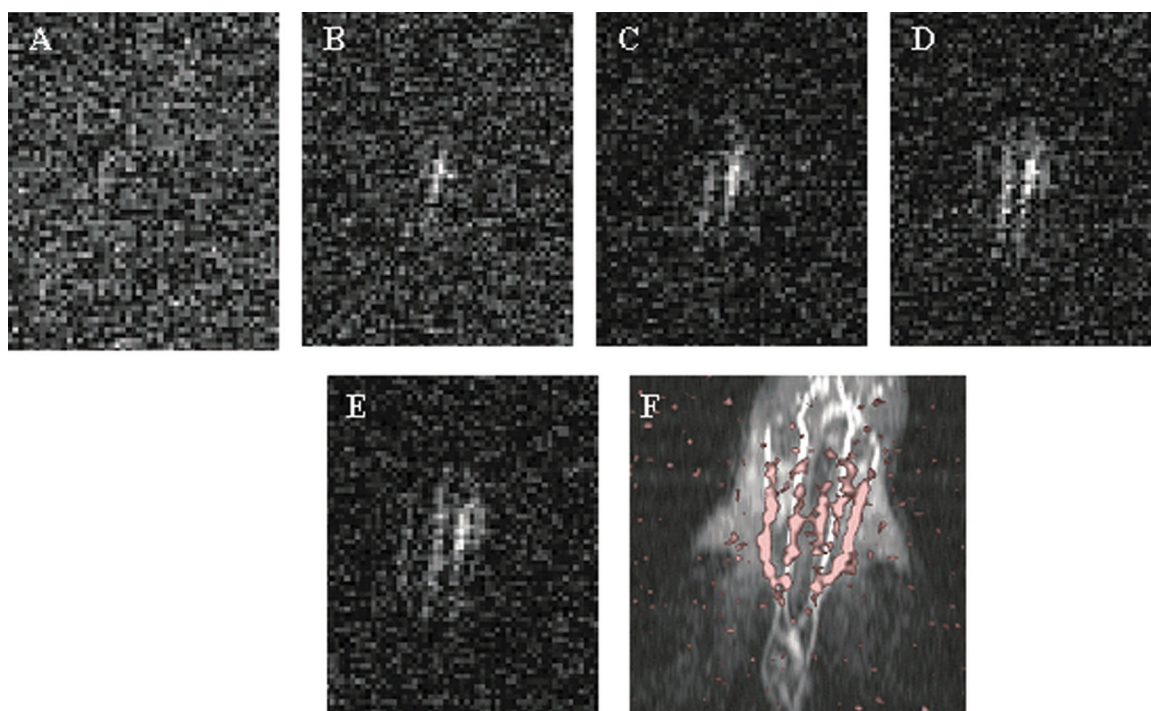
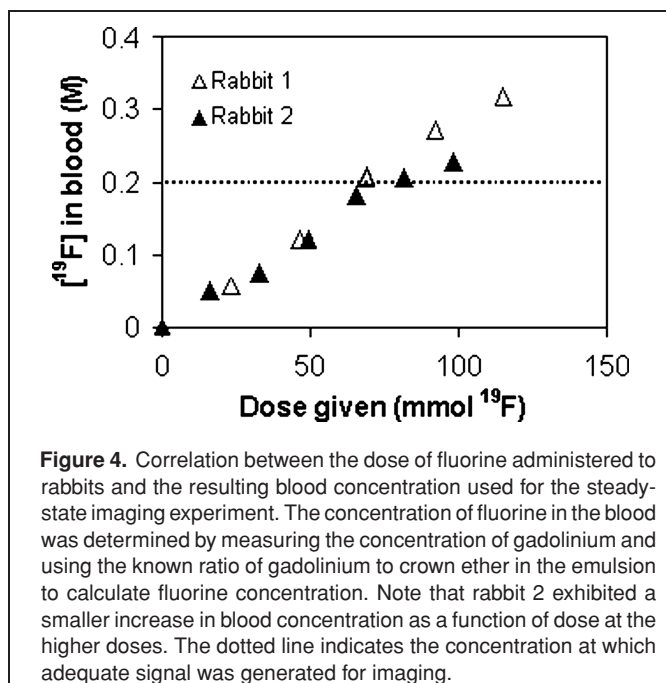


Figure 3. (A–F) Dynamic ^{19}F images of crown ether (CE) nanoparticles injected via a catheter into the left carotid artery of a live rabbit. Panel A shows the lack of detectable signal prior to the injection, while B–E show the accumulation of signal during the injection. F, Overlay of the ^{19}F signal from a longer scan onto a MIP of a time of flight angiography. Note the co-registration of the ^{19}F signal with the vessels in the neck.



higher signal from the vessels (Fig. 5, B–C). The second rabbit required a larger number of doses (6) for the same signal level obtained for the first rabbit (Fig. 5, D–E). The blood testing indicated that this rabbit experienced a smaller increase in serum

^{19}F for each dose as compared to the first rabbit (Fig. 4), likely a function of the different blood distribution volumes in each rabbit. After the sixth dose, however, a ^{19}F sagittal projection image of the neck did allow visualization of two vessels (Fig. 5, E), which matched those observed in a MIP of a phase contrast angiography (PCA) scan in the same orientation (Fig. 5, F). This approach provided a quantitative measure of the required blood concentration of ^{19}F to allow visualization using our system.

DISCUSSION

This work examines the concept of using ^{19}F CMR in conjunction with a perfluorocarbon nanoparticle contrast agent for angiography at clinical field strengths. While others have demonstrated the use of perfluorocarbon nanoparticles for imaging and oxygen mapping, this is the first demonstration of imaging of small vessels at clinical field strengths with sufficient temporal resolution to view the first pass injection through a catheter. Furthermore, we were able to image the steady-state blood signal from the nanoparticles delivered noninvasively at moderate doses, corresponding to 1.5–2.5 mL emulsion per kg body weight (or, equivalently, 0.5–0.9 g perfluorocarbon per kg). This dosage is well within the “absolute no effect dose” of 2.7–9 g PFC/kg determined using other PFC blood substitute emulsions (21). This nanoparticle contrast agent is constrained to the vasculature due to its size, making it an ideal candidate for

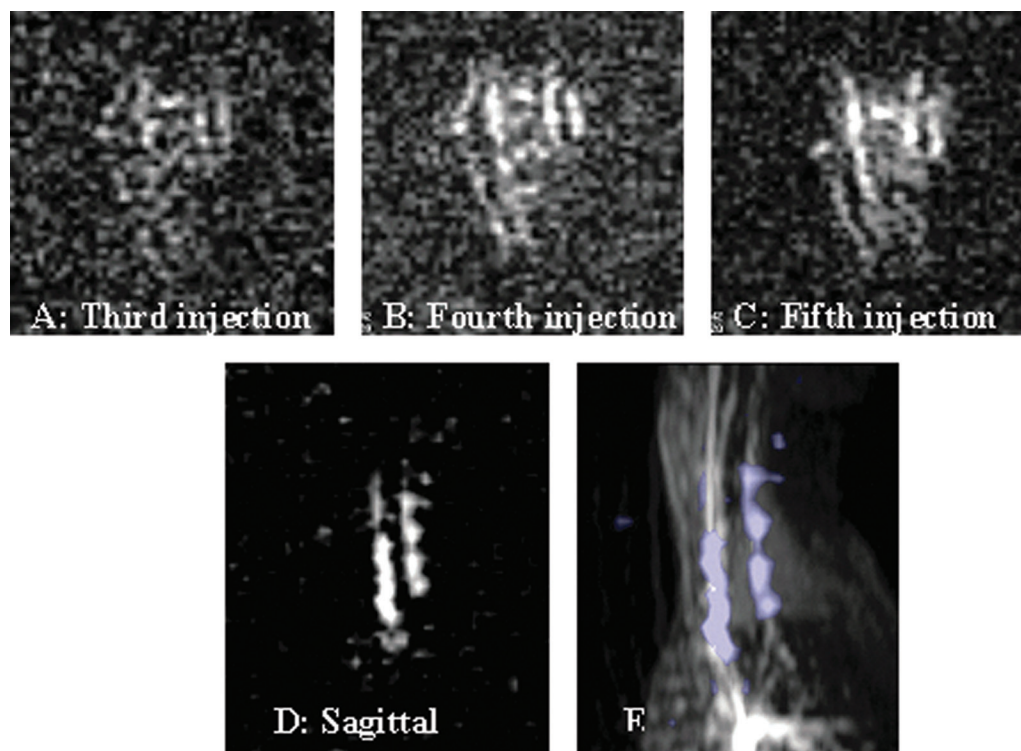


Figure 5. (A–C) ^{19}F coronal projections of the rabbit neck acquired after each systemic injection of the fluorinated contrast agent. **D**, A ^{19}F sagittal projection through the neck of the second rabbit after the sixth dose of particles. **E**, Overlay of the ^{19}F image in **E** (blue) onto a sagittal MIP of a ^1H phase contrast angiography scan in the same rabbit.

luminal imaging. In conjunction with the ability to use ^1H CMR for plaque characterization, this system could provide another tool for evaluating atherosclerosis using CMR.

The use of fluorine contrast agents for CMR is not a new concept, although the current demonstration of contrast angiography with nanoparticles under first pass and steady state imaging conditions indicates the potential for novel clinical applications. The development of fluorinated blood substitutes more than 30 years ago spurred interest in ^{19}F CMR because large amounts of fluorine could be safely injected into living tissue (21). The perfluorocarbon compounds themselves are biologically inert, and clearance of these emulsions from the blood is mediated by the reticuloendothelial system (macrophage uptake in the liver and spleen, primarily), resulting in a half-life of 4 to 10 hours depending heavily on particle size. After digestion of the lipid coat, the PFC can travel to the lungs via circulating lipid carriers, where it is vaporized and excreted. Ultimate clearance of the PFC from the body depends upon the lipophilicity, vapor pressure, and molecular weight of the particular perfluorocarbon. It was also discovered that these compounds exhibited a unique property: their longitudinal relaxation time (R_1) varies linearly with the partial pressure of O_2 to which they were exposed (10). This created an impetus for further studies and resulted in a variety of techniques to noninvasively map the oxygen concentration in tumors (12, 13, 22), heart (23), lungs (24, 25), and liver (26). Other studies used perfluorocarbons for general imaging of various organs, including the bowel (11, 27).

These approaches have met with some success, as indicated by the number of prior publications. However, significant hurdles remained for clinical application. First, many of the perfluorocarbons in use have a complicated ^{19}F nuclear magnetic resonance (NMR) frequency spectrum due to the presence of fluorine atoms with different chemical environments within the molecule. Compared to ^1H NMR, ^{19}F manifests larger chemical shifts such that the peak splitting caused by the shielding is not easily recombined into a single signal. As frequency is used as an indication of position in CMR, this translates into “ghosting” of the image and inaccurate positioning for slice selection. For simplicity and ease of demonstration, we were able to eliminate this problem in our study by using a perfluorinated crown ether structure. Perfluoro-15-crown-5 ether is ideal in that all of its fluorine atoms are chemically equivalent, so that all 20 atoms contribute to the image signal without the requirement for special imaging or image processing strategies. However, this technique could be applied using other perfluorocarbons with the use of narrow-bandwidth excitation (11, 15) or spectral deconvolution techniques (28, 29).

Despite the many advantages to using fluorine for generating CMR signal, the largest impediment is the sensitivity due to low concentrations. To overcome the inherently low signal available with fluorine CMR, earlier reports used some combination of high field strengths (12, 26, 30), large doses ($\sim 50\%$ of blood volume replaced) (11), and/or long scan times, all of which compromise clinical imaging applications. On the contrary, the current demonstration of fluorine angiography utilized far smaller doses of nanoparticles that would be much more practical for

clinical application, especially under conditions of steady state imaging. The required concentration of fluorine in the blood to allow visualization of the vessels was 0.2 M, or approximately a factor of 400 less than the typical concentration of ^1H in living tissues. The high level of signal generated with such a low concentration was obtained with the use of a recently implemented (5–7 years) “balanced” gradient echo imaging technique, which allows for rapid scanning and higher signal levels than obtained with any other sequence to date (31). By fully compensating for the dephasing effects of the read-out gradient, this pulse sequence is able to refocus “left-over” magnetization after the end of a pulse train, unlike other common sequences. In addition, the maximum signal obtained occurs when the sample of interest manifests comparable T_1 and T_2 times. Perfluoro-15-crown-5 ether is characterized by a very high ratio of T_2/T_1 relaxation time at 1.5 T, which renders this sequence particularly suited for fluorine angiography. The surprising amount of signal observed at 1.5 T with only modest amounts of fluorinated nanoparticles delivered intravenously lends credence to the prospect for noninvasive fluorine angiography.

The selection of image acquisition parameters in this paper was based on a simple and clinically relevant scan sequence, one that balances image and temporal resolution (thus, minimizing TR and TE), while maintaining adequate signal-to-noise. Examination of various permutations of multiple competing imaging parameters toward optimizing these characteristics could provide further improvements to this technique for fluorine angiography. In this study, certain factors complicated this analysis, including the use of surface coil for transmission and receive, which prevents accurate flip angle determination. Full characterization of relaxation parameters is also very important for sequence optimization. However, perfluorocarbon relaxation parameters are highly dependent upon oxygenation status, which varies *in vivo* as compared to ambient air, and also varies from arteries to veins, and with temperature. A rigorous examination of these factors could lead to improvements in sequence performance for this application.

Other techniques for imaging of the vessel lumen with CMR are phase contrast (PC) or time-of-flight (TOF) angiography, both of which utilize special ^1H imaging techniques to maximize the signal from flowing blood (32). While the final images are very useful and give quantitative information regarding flow, the image quality generated with these techniques is highly dependent upon the parameters chosen (for instance, the velocity encoding gradient amplitude) and adequate suppression of background signal, or so-called stationary spins. Scan times for these sequences can also be several minutes in duration, which can limit their utility in some situations. Contrast enhanced CMR angiography with gadolinium overcomes many of these limitations in that pre and post contrast images can be subtracted to suppress the background tissue signal. However, the gadolinium rapidly extravasates, requiring very fast scanning, thus limiting its utility for imaging more tortuous vessels and areas where motion is problematic, such as the coronaries.

Alternatively, perfluorocarbon nanoparticles might provide an unambiguous signal from the vessel lumen under steady state

imaging conditions without such requirements. To date, perfluorocarbon contrast agents and hyperpolarized gases have been the only intravascular contrast agents developed for CMR that can be used to generate images of the vasculature with no background signal from surrounding tissues (33). However, the perfluorocarbon particles do not require expensive specialized machinery for production and can be used “off-the-shelf.” Furthermore, hyperpolarized gases cannot be used under steady state blood concentration conditions since the signal dissipates rapidly after injection due to fast relaxation. The imaging methods evaluated in this work may ultimately allow estimation of lumen diameter in much the same way that traditional angiography is used.

Limitations

Although the *in vitro* and *ex vivo* fluorine signal levels were very high in the present study, the *in vivo* application will require further refinement before this technique can be tested clinically. One potential method for increasing the signal would be to use a more homogenous and sensitive RF coil such as a quadrature or phased-array coil. Secondly, the interaction of the gadolinium on the particles with the fluorine in the core should also be examined in future studies to ensure that it is not interfering. Methods for quantifying crown ether amounts directly are currently under development, which would eliminate the need for the gadolinium in studies similar to this one. Finally, the effect of oxygenation in the arteries and veins imaged in this study was not taken into account with regard to fluorine signal intensity and may affect interpretation of the images. This could be used as an advantage for differentiating arteries from veins in future studies.

CONCLUSIONS

This work demonstrates the use of perfluorocarbon nanoparticle contrast agents for ^{19}F CMR angiography using a rapid scanning technique. We were able to demonstrate the high level of signal obtained with this system using *in vitro* and *ex vivo* samples, as well as demonstrate *in vivo* imaging of rabbit carotid arteries both during direct injection with a catheter and after noninvasive intravenous dosing. With further developments and refinement, this technique may compliment the use of CMR for noninvasive imaging of vessel lumens and identification of atherosclerotic plaque.

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