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Fluorine-containing nanoemulsions for MRI cell tracking

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Abstract

In this article we review the chemistry and nanoemulsion formulation of perfluorocarbons used for *in vivo* ¹⁹F MRI cell tracking. In this application, cells of interest are labeled in culture using a perfluorocarbon nanoemulsion. Labeled cells are introduced into a subject and tracked using ¹⁹F MRI or NMR spectroscopy. In the same imaging session, a high-resolution, conventional (¹H) image can be used to place the ¹⁹F-labeled cells into anatomical context. Perfluorocarbon-based ¹⁹F cell tracking is a useful technology because of the high specificity for labeled cells, ability to quantify cell accumulations, and biocompatibility. This technology can be widely applied to studies of inflammation, cellular regenerative medicine, and immunotherapy.

IN VIVO CELL TRACKING USING MRI

Clinical Need

The persistence of complex, medical conditions, such as autoimmune diseases, cancer, and neurodegenerative diseases, has prompted the development of cellular therapies. Cellular therapy is the treatment of disease using therapeutic cells that have been derived from the patient, a matched donor, other organisms, or immortalized cell lines. Often these cells are sorted, multiplied, pharmacologically treated and/or genetically transformed in culture prior to infusion or transplantation into a patient. Therapeutic cells have the potential to accomplish complex tasks such as the regeneration of tissues, for example replacing cartilage^{1,2} or brain cells,³ or to re-program a patient's immune system, for example, to elicit an anti-tumor response.^{4,5} A common need for emerging cellular therapies is a non-invasive way to image the behavior and movement of cells following injection or transplantation into the subject. The ability to non-invasively monitor movement and accumulation of therapeutic cells would enable clinicians to determine whether cell delivery has occurred in the appropriate location, and whether—and to what degree—transplanted cells have reached their therapeutic location for each patient. *In vivo* cell tracking is potentially a powerful tool, providing critical feedback regarding the optimal routes of delivery and therapeutic doses for individuals. This feedback may come in the form of observed cell trafficking patterns and may also provide improved management of serious cell transplant side-effects, such as graft-versus- host disease.

Cell Tracking by MRI

MRI is a widely used clinical diagnostic tool because it is non-invasive, has no tissue depth penetration limitations, and provides contrast among soft tissues at reasonably high spatial resolution. Many laboratories have investigated the feasibility of using conventional ¹H MRI

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to visualize immune cells, stem cells, and other cell types. In certain studies, cultured cells are rendered magnetically distinct *ex vivo* via the internalization of paramagnetic contrast agents, for example, using nanometer- or micron-sized superparamagnetic iron oxide (SPIO) particles.^{6–11} There are also numerous studies where iron oxide particles have been used to label macrophages *in vivo* (i.e., *in situ*) upon direct injection (e.g., see reviews^{12–16}). On a more limited basis, perfluorocarbon (PFC) nanoemulsion formulations have also been used for ¹⁹F MRI cell tracking^{17–20} as an alternative to SPIO agents. PFC nanoemulsions have been shown to label monocytes and macrophages *in situ* when injected intravenously and provide positive signals at sites of inflammation in animal models.^{20,21}

Recently, ¹⁹F cell tracking methodologies have been developed that rely on *ex vivo* cell labeling with PFC. In this approach, cells of interest are labeled with a PFC nanoemulsion, injected or transplanted into a subject and the cell migration is monitored *in vivo* using spin-density weighted ¹⁹F MRI. Figure 1 shows the overall scheme of this methodology. The key advantage of this platform is that the ¹⁹F images are extremely selective for the labeled cells i.e., there is negligible background signal from the host's tissues.¹⁹ Furthermore, *ex vivo* labeling allows for tracking of phenotypically-defined cell types of interest.

The detection of PFC labeled cells using ¹⁹F MRI (or MRS) is fundamentally different from prior methods for labeling cells with paramagnetic agents. In the case of the latter, one detects the presence of the paramagnetic agent indirectly via its effect on T₁, T₂, and/or T₂* of surrounding protons in mobile water. In contrast, the PFC reagent acts like a 'tracer', and ¹⁹F magnetic resonance directly detects the density of ¹⁹F spins contained in the PFC droplets inside the cells.

The first proof-of-principle demonstration of *in vivo* ¹⁹F cell tracking using *ex vivo* cell labeling was published in 2005;¹⁹ in these studies PFC labeled mouse dendritic cells (DCs) were imaged. In follow-up studies, our laboratory has focused on labeling and tracking T cells in the context of following early inflammatory events in the non-obese diabetic (NOD) mouse model.²² Other laboratories have also used ¹⁹F MRI methodologies to visualize *ex vivo* labeled stem/progenitor cells *in vivo*.¹⁷ These examples demonstrate the breadth of possible applications of ¹⁹F MRI cell tracking.

In this article we describe the challenges and solutions for the design and synthesis of effective ¹⁹F MRI reagents for *ex vivo* cell labeling using PFC. We will also briefly discuss recent examples where these ¹⁹F cell tracking methodologies have been applied to animal models.

PERFLUOROCARBONS IN ¹⁹F MRI

Physicochemical Properties

PFCs have been studied for more than 40 years and are among the most chemically and biologically inert organic synthetic molecules ever produced.²³ The exceptionally strong carbon–fluorine covalent bond and the strong electron withdrawing effects of fluorine are responsible for the unusual physicochemical properties of PFCs. These molecules are highly hydrophobic, and in most cases significantly lipophobic. Due to the low polarizability of the fluorine atom, PFCs exhibit very low Van der Waals forces resulting in very low intermolecular cohesion, low surface tension, and high vapor pressure. These unusual physicochemical properties explain the ability of PFCs to dissolve oxygen, carbon-dioxide and nitrogen. Sharts and Reese²⁴ have shown that in emulsified linear perfluorocarbons, the number of fluorines directly correlates to its ability to solubilize oxygen. The first biological applications of PFCs exploited the high oxygen solubility. In 1966, Clark and co-workers demonstrated that mice could survive for 24 h while submersed in fluorocarbon liquid saturated with oxygen.²⁵ High

hydrophobicity and substantial lipophobicity of PFCs, and the consequent high tendency to segregate from the surrounding environment, are partially responsible for PFCs being biological inert and the absence of toxicity *in vivo* even at very high doses.

Perfluorocarbons as ^{19}F MRI Tracers

PFCs are a class of molecules that are potentially highly useful for intracellular MRI tracer applications. Fluorine has very low background biological abundance and is a sensitive atom for nuclear magnetic resonance spectroscopy or imaging. Compared to ^1H , the gyromagnetic ratio of ^{19}F differs by only about 6% and the relative sensitivity is 0.83. PFCs have a highly stable carbon–fluorine bond. There are no known enzymes that metabolize fluorocarbons *in vivo*,²⁶ and they do not degrade at typical lysosomal pH values.^{27,28} The ^{19}F NMR line shape and chemical shift of PFC is not altered within cells.¹⁹ PFCs are both lipophobic and hydrophobic and do not incorporate into cell membranes.^{27,28} Clearance of PFC agents from the body occurs via the reticuloendothelial system and exhalation through the lungs.²⁹

The utility of PFCs as MRI reagents is highly dependent on the chemical structure. Overall, optimal reagents for ^{19}F MRI applications should satisfy several design criteria, including: (i) chemical stability, (ii) a simple ^{19}F NMR spectrum, ideally having a single, narrow resonance to maximize sensitivity and prevent chemical shift imaging artifacts, (iii) a short ^{19}F T_1 and long T_2 to minimize data acquisition time.

A structural survey of PFCs used for MRI is shown in Figure 2. These structures can be classified into five groups: (a) aromatic and unsaturated PFCs (hexafluorobenzene, trans-1,2-bis(perfluorobutyl)-ethylene), (b) saturated linear PFCs (PFOB, PFOA, perfluorononane), (c) saturated ring system PFCs (perfluorodecalin, perfluoroperhydrophenanthrene), (d) perfluoroamines (FC-43) and (e) perfluoroethers and polyethers [PTBD, perfluoro-15-crown-5 ether (PCE), PFPE, FBPA]. From the structures shown in Fig. 2, only a few PFCs have been used for MRI cell tracking (PFPE, PCE, perfluorodecalin, PFOB), and of these, two have a large number of NMR-equivalent fluorine atoms (PCE and PFPE). Specific PFC examples and their properties as MRI tracers are discussed in detail below.

Perfluorooctyl bromide [(PFOB) perfluorobron, Figure 2(b)], a linear PFC, was one of the earliest applied as a ^{19}F MRI tracer.³⁵ It has been used for oxygen sensing,^{36,37} imaging tumors,³⁸ atherosclerotic plaques,³⁹ and cell tracking.¹⁷ PFOB is hydrophobic and, unlike most PFCs, shows a small but finite lipophilicity due to the covalently-bound bromine. The limited membrane solubility localizes PFOB to the center of lipid bilayers.⁴⁰ The PFOB ^{19}F NMR spectrum consists of eight peaks, one for each CF_n moiety; its use for cell tracking is non-ideal in terms of sensitivity and requires the use of a frequency selective MRI pulse sequence to limit chemical shift artifacts, for example, by incorporating presaturation RF pulses on undesired resonance peaks prior to imaging.

Perfluorotributylamine [(FC-43), Figure 2(d)], has been used for *in vivo* oxygen sensing in porcine models.⁴¹ FC-43 also shows multiple ^{19}F peaks, and thus its use for imaging is as challenging as in the case of PFOB. Perfluorinated ether PTBD (Figure 2(e)) was introduced as an improvement for oxygen sensing by ^{19}F MRI. It has a ^{19}F spectrum less complex than PFOB or perfluorotributylamine, with 12 equivalent fluorines from the CF_3 groups. PTBD can be imaged without the use of MRI pulse sequences that suppress chemical shift artifacts. Overall, PTBD shows an increase in sensitivity of 17% over PFOB at the same molar concentration.⁴²

Further improvement in MRI sensitivity is possible with perfluorinated ether PCE (Figure 2 (e)), a chemically and biologically inert macrocycle with 20 equivalent fluorine nuclei having a single resonance at -92.5 ppm. This molecule satisfies most of the theoretical ^{19}F MRI

reagent design criteria (i and ii). Oxygen tightly coordinates with PCE, forming a complex that is detectable by mass spectrometry⁴³ and significantly shortens the ^{19}F T_1 . This effect has been exploited in local oxygen sensing studies in tumors^{44–46} and in the brain.⁴⁷ PCE was also used for MRI detection of atherosclerosis in animal models³⁹ and for *in vivo* cell tracking.^{17,34,48}

Recently, PFPE (Figure 1(d)), a linear perfluoropolyether polymer, was introduced to meet all of the above design criteria (i–iii) for a ^{19}F MRI cell tracking reagent.^{22,49} The linear PFPE has a simple ^{19}F NMR spectrum^{22,34,50} with >40 equivalent fluorines from $\text{CF}_2\text{CF}_2\text{O}$ monomer repeats and the major resonance is close to that of PCE (–91.5 ppm). Linear PFPEs, as well as PCE, are synthesized from analogue hydrocarbons by direct fluorination.^{50,51} Recently, linear PFPE was first used for ^{19}F cell tracking and quantification *in vivo* in a mouse diabetes model.²²

Fluorous phase synthetic approaches^{47,52} take advantage of linear PFCs tendency to behave as their own liquid phase when mixed with water, lipids or organic solvents.²⁶ Recently, fluorous phase synthetic strategies were used for chemical modification of linear PFPEs resulting in fluorescent ‘blended’ PFPE amides³⁴ (FBPAs, Figure 2(e)). The conjugation approach exploits the high reactivity of PFPE alkyl esters (Figure 2(d), where $\text{R} = \text{C}(\text{O})\text{OMe}$) to nucleophilic addition of primary or secondary amines.⁵³ Fluorescent dye is directly conjugated to PFPE via aliphatic amino linker, and unconjugated dye is removed by fluorous phase liquid-liquid extraction.³⁴ FBPAs behave as a unique fluorous oil phase during nanoemulsion formulation and retain their fluorescent properties *in vivo*.³⁴ Application of FBPAs to cell tracking and ^{19}F MRI is discussed in Section 4.

PFC NANOEMULSION FORMULATION FOR CELL LABELING

PFCs do not mix with cell membranes and would not enter cells unless formulated into a biocompatible nanoemulsion or microemulsion. A significant body of work on formulating stable nanoemulsions has been reported in the context of developing vascular imaging agents or artificial blood substitutes.^{23,26,54} In these applications, nanoemulsions have to be highly stable in vascular circulation for many hours. The surfactants used in these types of formulations should promote stability in the blood stream and possibly passive or active targeting to macrophages *in situ*. However, these are not necessarily desirable design features for PFC nanoemulsions used in *ex vivo* cell labeling.

A PFC nanoemulsion for cell tracking must efficiently and safely label target cells *ex vivo* before administering to the subject by injection. Empirically, safe loading levels of PFC are found to be on the order of 10^{11} – 10^{12} fluorine atoms per cell without overt toxicity.^{22,34,48,49} Ideally, a nanoemulsion for ^{19}F MRI cell tracking using *ex vivo* labeling should satisfy the following design criteria: (1) small droplet size, ideally <200 nm, (2) low polydispersity index (PDI), ideally less than 0.2, (3) maximum fluorine-to-surfactant ratio in order to minimize the amount of MRI-inactive material delivered inside the cell, (4) a surface that promotes cell membrane interaction and/or cellular uptake, (5) long-term intracellular retention for longitudinal MRI tracking, (6) long shelf-life, ideally >1 year, (7) low toxicity to cells and tissues, (8) does not modify phenotype, morphology or biological function of the labeled cells, and (9) PFC nanoemulsions should be able to label a wide range of cell types.

PFC Nanoemulsions

Nanoemulsions are kinetically stable emulsions with a droplet size typically between 20 and 500 nm. Unlike microemulsions, which can form spontaneously, nanoemulsions require high energy processing, such as high pressure, high shear homogenization (e.g., microfluidization)⁵⁵ or sonication. Nanoemulsion formation usually does not require high amounts of surfactant and can be stabilized by steric effects.⁵⁶ The most common degradation mechanism for

nanoemulsions is Ostwald ripening, a molecular diffusion phenomena that results in a gradual growth of the larger particles at the expense of smaller ones.^{56,57} Nanoemulsion degradation via Ostwald ripening represents a major problem in formulating stable PFC nanoemulsions.^{58–60} The choice of surfactant, emulsification technique and PFC structure can significantly slow down but cannot fully prevent Ostwald ripening. PFC structure significantly affects the Ostwald ripening rate.^{58,60} A recent study has shown that perfluorodecalin (Figure 2(c)) forms smaller nanoemulsion droplets and exhibits a lower droplet growth rate due to Ostwald ripening than *n*-perfluorohexane.⁵⁸ Molecular diffusion is more likely to occur in *n*-perfluorohexane than in perfluorodecalin emulsions and can be associated with the larger solubility and diffusion coefficients of *n*-perfluorohexane in external phase. These findings are directly relevant to hydrocarbon nanoemulsion degradation studies, where the molar volume of the hydrocarbon inversely correlates to the nanoemulsion degradation rate.⁶¹ Branched and longer chain PFCs give more stable emulsions under the same conditions compared to short chain PFCs. Kabalnov⁶⁰ suggests that the emulsion stability depends, to a larger extent, on the perfluorocarbon structure rather than the emulsifier. However, this is far from being a rule and surfactants can play a significant role in emulsion stability. Possible methods to decrease Ostwald ripening, for example, include the addition of water-insoluble oils such as a long triglyceride to the nanoemulsion⁶² or the use of longer chain perfluorocarbons.⁶³

Emulsifiers and Surfactants for PFC Nanoemulsions

In addition to the PFC structure, the choice of appropriate emulsifier is of great importance to stabilize the nanoemulsion and to render it biocompatible. Suitable emulsifiers for preparing nanoemulsions for cell labeling must be non-toxic, chemically stable, and help to reduce the characteristically large interfacial tension of perfluorocarbons in the aqueous phase. Only a few surfactants and emulsifiers have been proven successful in stabilizing PFC colloid dispersions in water. Below, we discuss the most commonly employed emulsifiers for preparing PFC nanoemulsions suitable for MRI. Phospholipids (e.g., egg yolk phospholipids, EYP) and pluronics (e.g., poloxamer 188 or F68) are the most common emulsifiers.²³ The addition of a fluorinated surfactant to stabilize the nanoemulsion has also been suggested.⁶⁰ However, this approach is not suitable for stabilizing ¹⁹F MRI reagents, due to the potential for chemical shift artifacts caused by the fluorine in the surfactant molecules.

Lipids—One approach to formulate stable PFC nanoemulsions utilizes lipids and organic oils. Researchers have developed a PFOB nanoemulsions that were found to be useful in both ultrasound and MRI.^{17,18,64,65} These PFOB nanoemulsions used safflower oil and a lecithin-containing surfactant co-mixture. The nanoemulsion satisfied two requirements for stability: first, the emulsifiers were lipids, and based on Kabalnov,⁶⁰ the PFC nanoemulsions prepared with lipids are more stable, and second, the added safflower oil decreases the Ostwald ripening, as a hydrophobic additive. The resulting nanoemulsion had a low droplet size (224 nm) and a high PDI of 0.350, which is consistent with earlier findings^{58,62} that lipid based perfluorocarbon nanoemulsions contain heterogeneous droplet diameters. The same emulsification method was used to prepare PCE nanoemulsion, which resulted in similar droplet size (233 nm) and a significantly lower PDI (0.170). This shows that PFC structure does not have substantial effect on droplet size, but affects the nanoemulsion droplet PDI.

Non-ionic surfactants—One of earliest reported PFC nanoemulsion surfactants was Pluronic F68™ (BASF).²⁴ This molecule is a poly(ethylene oxide)–poly(propylene oxide)–poly(ethylene oxide) (PEO–PPO–PEO) tri-block non-ionic copolymer that is FDA approved for human use. F68 stabilizes the PFC oil droplets in aqueous phase mostly by steric effects.⁶⁶ Previous structural studies have shown that block polymers adsorb onto the interface between the colloid PFC and the aqueous phase and that this adsorption is dependent on the electrolyte concentration in the solution.^{67–69}

Recent studies describe the development of a novel, highly stable nanoemulsion prepared with linear PFPE, pluronic F68 and linear short chain polyethylenimine (PEI).³⁴ Figure 3(b) shows 125 day stability data by dynamic light scattering (DLS) at three different temperatures, and no change in nanoemulsion droplet size or PDI was observed. Further testing showed no signs of Ostwald ripening up to 14 months of follow up (data not shown). In this example we speculate that the high molecular weight linear PFPE is responsible for high nanoemulsion stability from Ostwald ripening degradation, aided by steric stabilization from the F68.

IN VIVO ¹⁹F MRI AND CELL TRACKING

Cell tracking by ¹⁹F MRI has the potential to accelerate studies of inflammation and regenerative medicine because it is non-invasive and has high specificity. Previously, ¹⁹F MRI of PFCs has been used for several, mostly extracellular applications.^{35,70–71} Various types of PFCs have long been contemplated²⁵ as oxygen carriers during surgery and as artificial blood substitutes,⁷¹ and thus a large volume of data exists on the toxicity profile and clearance pathways of these materials. In the above examples large doses of PFC are delivered intravenously. In contrast, ¹⁹F cell tracking applications using *ex vivo* cell labeling uses only a comparatively miniscule mass of the PFC agents (i.e., contained within the transferred cells).

PFC acts like a ‘tracer’ agent, and ¹⁹F magnetic resonance directly detects the density of ¹⁹F spins (nuclei) contained in the PFC droplets located inside the cells, which is generally of low concentration compared to the ¹H nuclei contained in the ~50 M mobile water. Thus, the signal-to-noise-ratio (SNR) of the ¹⁹F images will be significantly lower than that commonly observed for ¹H MRI. Paramount, however, is that one does not demand a high ¹⁹F SNR. Because there is negligible ¹⁹F background, any ¹⁹F signal detected is from labeled cells. Unlike ¹H anatomical imaging, where one relies on a high SNR to resolve detailed anatomy and organ definition, the ¹⁹F image only needs to detect localized pools of cells at arbitrarily low SNR; the ¹H image overlay provides the detailed anatomical context.

The ultimate sensitivity of the ¹⁹F MRI cell detection technique is not yet known and certainly depends on many technical details, such as the cell type, subject, and the imaging system used. From basic principles, it is straightforward to perform a ‘back of the envelope’ calculation of the practical limits of the number of PFC-labeled cells per voxel that can be detected. This is very conservatively estimated to be ~10⁵ cells per voxel using a conventional 1.5T clinical scanner.¹⁹ Empirical MRI phantom studies show that one can detect ~7,500 labeled T cells per voxel using *in vivo*-like acquisition parameters in a high-field MRI system.²² Overall, we believe the practical cell detection sensitivity is on the order of 10⁴–10⁵ cells per voxel clinically. In high field animal scanners, the sensitivity will be better than 10⁴ cells per voxel. Recent studies from Washington University¹⁷ support this view, where they imaged PFC-labeled stem cells implanted in a rodent on a 1.5T scanner in a short scan time (~7 min). Single cell imaging is not possible with the PFC labeling, as has been reported by several groups using SPIO agents.^{72,73} More importantly, MRI applied to cell tracking problems will demand unambiguous identification and robust quantification of regions where thousands of cells cluster. Overall, work remains to optimize the ¹⁹F MRI acquisition schemes for cell tracking.

Ideally PFC nanoemulsions can label a wide range of cell types causing no toxicity, or changes in phenotype, morphology or function, while providing sufficient intracellular ¹⁹F signal for MRI. Recent reports utilize PCE^{17,19} and PFOB nanoemulsions¹⁷ for cell tracking by ¹⁹F MRI. Cellular uptake mechanisms of PFC nanoemulsions have yet to be studied in detail. Our lab originally used¹⁹ a PCE lipid based nanoemulsion with a commercially available cationic lipid transfection reagent, achieving sufficient cell loading and adequate ¹⁹F MRI signal *in vivo* (Figure 4). In these studies, transmission electron microscopy shows nanoemulsion droplets as low-electron density spheroids within compartments consistent with vesicles.

More recent studies³⁴ report 'self-delivering' nanoemulsions that efficiently label non-phagocytic and phagocytic cells without the need of transfection reagents. The linear PFPE nanoemulsion labeled primary T cells while retaining their phenotype and were successfully imaged *in vivo* (Figure 5).

In vivo ¹⁹F MRI cell tracking is often not sufficient to follow cellular fate and cellular phenotype post-transfer. A dual fluorescent-MRI cellular labeling reagent is highly desirable for recovery and further study of cells post-imaging. The fluorescent moiety facilitates unambiguous identification and recovery of labeled cells from tissues. Fluorescence-based detection techniques (e.g., microscopy and immuno-cytochemistry) can provide confirmation of the MRI cell tracking results in biopsied tissues. One such reagent was developed recently, where linear PFPEs were conjugated directly to fluorescent dyes.³⁴ Following the *in vivo* imaging, fluorescence microscopy and FACS analysis were performed on the labeled T cells. Figure 6 shows confocal microscopy of labeled DCs and T cells with BODIPy-TR PFPE nanoemulsion. These new dual mode reagents open new avenues for imaging as well, including, for example, the use of live animal fluorescence imaging.

CONCLUSIONS AND FUTURE DIRECTIONS

In this review we have discussed the overall usefulness of PFC nanoemulsions as reagents for ¹⁹F cell tracking. We described the design and preparation of these reagents through specific examples reported to date. Introducing additional imaging and detection modalities to PFC nanoemulsions opens new avenues for MRI cell tracking and promises to vastly improve current technology. Although the biomedical application of these new technologies are still nascent, we believe that in the future it will be possible to translate ¹⁹F cell tracking into the clinical arena.

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FIGURE 1.

Cellular MRI using PFC nanoemulsion technology. PFC nanoemulsion is added to cultured cells that have been harvested from a subject or an engineered line. The labeled cells are transplanted into the subject and imaged using ^{19}F and ^1H MRI in the same imaging session. The registered images are overlaid, yielding an image of the labeled cells in their anatomical context.

FIGURE 2. Survey of PFCs for potential MRI applications.^{20,22,30–34}

FIGURE 3.

DLS analysis of a linear PFPE nanoemulsion emulsified with F68 and PEI. Panel A displays the diameter distribution by light intensity, and B shows the diameter and PDI (error bars) followed over time at three different storage temperatures: 4 (■), 25 (●), and 37 (▼) °C. (Adapted from Ref 34).

FIGURE 4.

In vivo MRI using PFC with dendritic cells (DCs) in mouse. Images of the labeled cells (i.e., ^{19}F images) are displayed on a 'hot-iron' intensity scale, and the anatomical (^1H) images are shown in grayscale. The three panels on the far left are a mouse quadriceps after intramuscular injection of DCs (asterisk indicates injection site). (a) ^{19}F and ^1H images (from left to right) and a 'composite' $^{19}\text{F}/^1\text{H}$ image. (b) The composite image of DC migration into the popliteal lymph node following a hind foot pad injection. (c) Composite image through the torso following intravenous inoculation with labeled DCs. Cells are apparent in the liver, spleen, and weakly in the lungs. (Data taken from Ref 19).

FIGURE 5.

In vivo MRI of labeled T cells in the mouse model. The ^{19}F image (pseudo-color) shows a localized accumulation of T cells labeled with PFPE nanoemulsion in lymph nodes and the grayscale underlay is an anatomical ^1H image. Panels (a) and (b) display two consecutive 2 mm thick slices through the torso, and for anatomical orientation the kidneys (K) and gut (G) are noted. During imaging, the mouse was anesthetized with a ketamine/xylazine cocktail, connected to a mechanical ventilation apparatus, acquisitions were cardio-respiratory gated, and body temperature was regulated at 37°C . Data were collected for both ^{19}F and ^1H in a single imaging session. (Adapted from Ref 34).

FIGURE 6. Dual-mode reagents for ^{19}F MRI and fluorescent detection. Confocal microscopy of (a) labeled mouse DCs (b) and primary T cells labeled with FBPA (red). (Adapted from Ref 34).