Enriching Fluorine Nanoparticles with Saturated Phosphoethanolamines to Improve Dendritic Cell Detection by ^{19}F Magnetic Resonance In Vivo

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Target audience: MR scientists, clinical scientists, immunologists, cell biologists

Introduction and Purpose: Magnetic resonance (MR) methods to detect and quantify fluorine (^{19}F) nuclei provide the opportunity to study the fate of cellular transplants *in vivo*. Cells are typically labeled with ^{19}F nanoparticles (NPs), introduced into living organisms and tracked by ^{19}F MR methods[1,2]. Background-free imaging and quantification of cell numbers are amongst the strengths of ^{19}F MR-based cell tracking but challenges pertaining to signal sensitivity and cell detection exist. In this study we aimed to overcome these limitations by manipulating the lipid composition of ^{19}F NPs in order to promote their uptake by dendritic cells (DCs). We studied the uptake mediating potential of phosphatidylethanolamines (PE) on DC considering their significance in biological membranes as well as the power of PE analogs to promote cellular uptake of transfection and drug delivery systems[3].

Methods: NPs with high ^{19}F content were prepared using Perfluoro-15-crown-5-ether (PFCE, Fluorochem, UK) emulsified in Pluronic F-68 (Sigma-Aldrich) via direct sonication[2] or by incorporating PFCE in PEG2000-DSPE liposomes. Pluronic-based NPs were enriched with varying concentrations of the aminophospholipids 1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine (DPPE) and 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoethanolamine (POPE) (both from Avanti Polar Lipid, Inc., Alabaster, AL) by further sonication. Bone-marrow derived DCs were labeled overnight with ^{19}F NPs and then washed thoroughly with PBS. To monitor ^{19}F uptake, ^{19}F spectroscopy was performed on fixed cells using a ^{19}F tuned loop coil designed in-house[2] for signal transmission and reception on a 9.4 T animal MR scanner (Biospec 94/20 USR, Bruker Biospin). Global ^{19}F spectroscopy signals were acquired using a 90° block excitation pulse. Voxel-based ^{19}F PRESS was used to study ^{19}F-labeled DCs. An NMR tube holder printed in-house was fitted in a ^{1}H/^{19}F dual-tunable RF body coil (Rapid Biomed, Würzburg, Germany). After B_0 shimming, ^{19}F spectra were acquired within a (5×5×5) mm^3 voxel: TR=1500 ms, TE=11.6 ms, number of repetitions=512, scan time=13 min. 3D balanced steady state free precession (bSSFP) MRI was employed to acquire ^{19}F signal in phantom and *in vivo* experiments. ^{1}H bSSFP: TR=6.7 ms, TE=3.3 ms, flip angle=30°, matrix=256×128×128, FOV=(5.8×2.9×5.8) cm^3, NEX=1, scan time=63 sec. ^{19}F bSSFP: TR=3.6 ms, TE=1.8 ms, FA=30°, matrix=64×32×32, FOV=(5.8×2.9×5.8) cm^3, NEX=64, scan time=16.85 min. Four bSSFP scans were acquired (using 0°, 90°, 180° and 270° phase cycling scheme) and combined by sum of squares. Animal experiments were carried out in accordance with the State Office of Health and Social Affairs Berlin (LaGeSo). ^{19}F-labeled DCs were administered intradermally (5×10^6) into C57BL/6 mice[4] and imaged 4h following injection. Anesthetized mice were imaged using the same ^{1}H/^{19}F RF body coil and 3D bSSFP pulse sequence as above. For quantification of the ^{19}F content within the lymph node (LN) regions, we also employed the same PRESS sequence after placing a (3×3×3) mm^3 voxel around the LNs.

Results: For Pluronic-based NPs we chose two PE analogs differing in their fatty acid (FA) hydrocarbon chains; POPE contains one unsaturated bond and DPPE contains only saturated bonds in its FA chains. For liposomal NPs, we also chose a PE consisting solely of saturated bonds (DSPE), bound to polyethylene glycol-2000 (DSPE-PEG2000) (**Figure 1**). Pluronic-based NPs enriched with PE polymers appeared to be taken up more efficiently by DCs than basic Pluronic nanoparticles and DSPE–PEG2000 liposomes as determined by the increase in cellular ^{19}F signal (**Figure 2**). POPE enrichment already resulted in an increased ^{19}F signal compared to the basic nanoparticle formulations and DSPE–PEG2000 liposomes. When we employed DPPE to enrich the nanoparticle shell, we observed an even stronger enhancement in ^{19}F signal, which equates to an order of magnitude increase from 10^{12} (basic formulations) to 10^{13} (DPPE-enriched nanoparticles) ^{19}F spins per DC unit. Taking into account the dramatic increase in ^{19}F signal following DPPE enrichment we titrated PFCE amount from 40 µmol to 2 µmol per 10^7 DCs. Lower PFCE concentrations in DPPE ^{19}F NPs (5 µmol) gave a larger ^{19}F signal than 40 µmol PFCE label within basic ^{19}F NPs (data not shown). Using 5 µmol PFCE for both DPPE and basic ^{19}F NPs we next measured the ^{19}F signal in increasing numbers of DCs to determine the cell detection limit. The ^{19}F signal amplitude correlates with number of ^{19}F labeled cells for both groups but smaller numbers of DPPE-NP labeled DCs could be detected when compared to basic-NP labeled DCs (**Figure 3**). Following *in vivo* application we also observed that DPPE-NP labeled DCs gave a more prominent signal *in vivo* than the basic-NP labeled DCs (**Figure 4**).

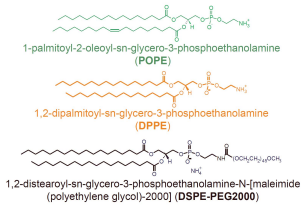

Figure 1: Selection of PEs used for enriching ^{19}F nanoparticles. Shown are the chemical structures of POPE, DPPE and DSPE-PEG2000.

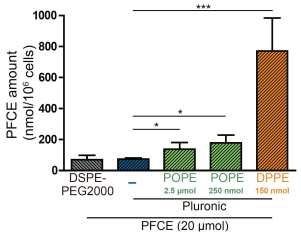

Figure 2: DCs were labeled with different ^{19}F nanoparticle preparations using a PFCE concentration of 20 µmol/10^7 cells. The PFCE amount per 10^6 cells was calculated using a 500 mM PFCE standard.

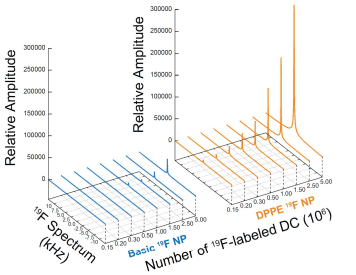

Figure 3: Lower number of cells detected by ^{19}F MRS when DCs are labeled with DPPE-enriched ^{19}F nanoparticles. Shown are ^{19}F spectral representations from different numbers of basic and DPPE-NP labeled DCs using 5 µmol PFCE/10^7 cells.

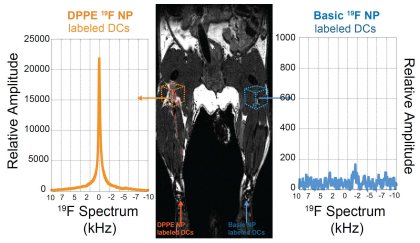

Figure 4: *In vivo* ^{19}F/^{1}H MRI and voxel-based ^{19}F MRS. DCs (10^7) were intradermally injected in C57BL/6 mice following labeling with DPPE-enriched ^{19}F NPs (left hind limb) or basic ^{19}F NPs (right hind limb). 3 hours later a (3×3×3) mm^3 voxel was placed around left and right popliteal LN and ^{19}F content measured using PRESS.

Discussion and Conclusions: By introducing DPPE to ^{19}F NPs we improved NP uptake by DCs; we show that DPPE incorporation increases intracellular ^{19}F signal in DCs by at least one order of magnitude compared to ^{19}F NPs devoid of DPPE. PEs constitute an integral part of biological membranes and have been employed as components of non-biological systems such as non-viral transfection agents[5] and as coatings of poorly soluble carbon nanotubes to promote cellular uptake[3]. In our study we provide one way of boosting ^{19}F signal per cell in order to overcome some of the limitations related to ^{19}F MR signal sensitivity. The boost in signal is ultimately necessary to detect and track cells in vivo.

References: 1. Ahrens ET *et al.* Nat Biotechnol 2005;23: 983-987. **2.** Waiczies H *et al.* PLoS One 2011;6: e21981. **3.** Antonelli A *et al.* Nanotechnology 2010;21: 425101. **4.** Waiczies H *et al.* J Vis Exp 2013;73: e50251. **5.** Felgner JH *et al.* J Biol Chem 1994;269: 2550-2561.