Influence of phospholipid enriched ^{19}F nanoparticles on fluorine uptake in dendritic cells

Stefano Lepore[1], Karl Sydow[2], Susanne Drechsler[1], Min-Chi Ku[1], Helmar Waiczies[1,3], Conrad Martin[1], Irene Schuetz[2], Bettina Purfürst[4], Margitta Dathe[2], Thoralf Niendorf[1,5], and Sonia Waiczies[1,5]

[1]Berlin Ultrahigh Field Facility (B.U.F.F.), Max Delbrück Center for Molecular Medicine, Berlin, Berlin, Germany, [2]Department of Medicinal Chemistry/Chemical Biology, Leibniz-Institut für Molekulare Pharmakologie, Berlin, Berlin, Germany, [3]MRI.Tools GmbH, Berlin, Berlin, Germany, [4]Electron Microscopy, Max Delbrück Center for Molecular Medicine, Berlin, Germany, Berlin, Berlin, Germany, [5]Experimental and Clinical Research Center (ECRC), Berlin, Berlin, Germany

Introduction: Studying the migratory behavior of immune cells is pivotal to better understand the development of immune-mediated pathologies such as autoimmunity. It is therefore of high relevance to develop methods for non-invasive and in vivo tracking of immune cells. It has been already demonstrated that fluorine (^{19}F) MRI enables spatio-temporal tracking of dendritic cells (DC) [1,2] *in vivo* and monitoring inflammation during the development of brain autoimmunity [3] using cells labeled with ^{19}F rich nanoparticles. The aim of this study is to overcome the ^{19}F sensitivity barriers by increasing the uptake of ^{19}F nanoparticles in DC. Therefore we explore the impact of aminophospholipids incorporated into the ^{19}F nanoparticle on uptake by DC and on labeling.

Methods: Four different formulations of nanoparticles with high fluorine content were prepared by emulsifying Perfluoro-15-crown-5-ether (PFCE, Fluorochem, UK) via direct sonication, using a ultrasonic homogenizer (Hielscher Ultrasonic GmbH). The PFCE–Pluronic formulation (**PFCE–Plur**) was prepared by emulsifying PFCE in Pluronic F-68 (Sigma-Aldrich). The PFCE–Pluronic–1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine (DPPE, Avanti Polar Lipid, Inc.) formulation (**PFCE–Plur–DPPE**), was prepared by emulsifying the PFCE-Plur particles further in phosphate/buffered saline (PBS) and DPPE (50 µl) using the same conditions. The same procedure was used to prepare the PFCE–Pluronic–Rhodamine-DPPE (**PFCE–Plur–Rhod-DPPE**) formulation. The PFCE–1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000] (PEG2000) particles (**PFCE–PEG2000**) were prepared by sonicating PFCE diluted in PBS (1:25) in PEG2000-DSPE. DC were prepared from bone marrow suspensions as previously described [4]. After 10 days in culture, DCs were labeled with the different nanoparticle preparations, overnight and then washed 3 times with PBS. To monitor the ^{19}F uptake within fixed cells by ^{19}F spectroscopy we designed a ^{19}F tuned loop coil [2] for signal transmission and reception on a 9.4 T animal MRI scanner (Biospec 94/20 USR, Bruker Biospin). The ^{19}F signal was acquired using a 90° block excitation pulse with 10 kHz bandwidth and the amplitude was calculated by performing a fast Fourier transformation (FFT) of the acquired free induction decay (FID). For electron microscopy, ultrathin plastic sections of the DC pellet were stained with uranyl acetate and lead citrate. Sections were imaged using a FEI Morgagni electron microscope and iTEM software. Dendritic cells fluorescence was studied using Laser Scanning Microscopy (LSM780, Carl Zeiss MicroImaging GmbH, Jena, Germany). For the migration assay two agarose spots containing chemokine CCL21 and two containing PBS were placed onto 35-mm glass dishes (MatTek). ^{19}F-nanoparticle labeled DCs were then introduced to the dishes. After 3 to 4 hrs, the number of DC under the spot was counted.

Results: The uptake of ^{19}F nanoparticles by DC was dependent on the lipid content in the different formulation. ^{19}F Spectroscopy of labeled DC revealed that nanoparticle formulations supplemented with PFCE–Plur–DPPE or PFCE–Plur–Rhod-DPPE are internalized more easily by DC versus the PFCE–Plur and PFCE–PEG2000 formulations (Fig. 1). For example the PFCE–Plur–DPPE formulation showed one order of magnitude improvement in uptake. In agreement with ^{19}F spectroscopy, EM and LSM showed that DCs labeled with aminophospholipid-enriched ^{19}F nanoparticles (Fig 2C and 2D) were heavily packed within cytosolic organelles (white arrows in Fig. 2) in comparison to the other two nanoparticle formulations (Fig. 2A and 2B). To assess the impact of the higher uptake of these modified nanoparticles on DC functionality, we performed a migration assay with cells labeled with increasing concentrations of PFCE–Plur or PFCE–Plur–DPPE nanoparticles. Figure 3 shows how the enhanced ^{19}F signal of PFCE-Plur-DPPE (Fig 3, open circles), as a result of an increased concentration of PFCE inside the cells coincided with a decreased mobility versus an increase in the labeling concentration. This drop in mobility was more pronounced when compared to PFCE-Plur particles labeled cells (Fig. 3, filled squares).

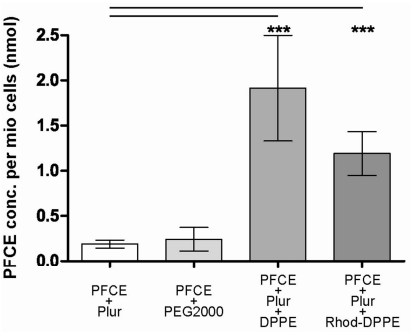

Figure 1 – PFCE concentration after labeling. Fixed DC were labeled with different nanoparticle formulations and then ^{19}F signal measured using ^{19}F spectroscopy and translated to an average ^{19}F concentration (nmols) per million cells, as shown.

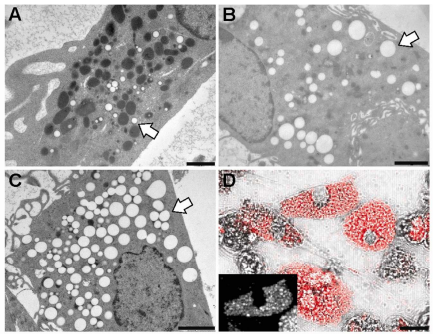

Figure 2 – EM (A, B, C) and LSM (D) images of labeled DC. DC labeled with PFCE–Plur (A), PFCE–PEG2000 (B), PFCE–Plur–DPPE (C) and PFCE–Plur–Rhod-DPPE (D). Size bars: 1µm (A), 2µm (B,C) and 10µm (D).

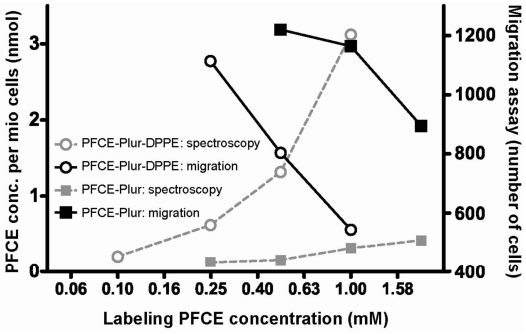

Figure 3 – Concentration-dependent labeling load and cell functionality. The graphs show the increased particle uptake (dashed line) and the decreased migration capability (continuous line) of DC labeled with PFCE-Plur-DPPE (open circle) compared to basic PFCE-Plur formulation (bold square).

Discussion: We demonstrated that the introduction of aminophospholipids into the structure of ^{19}F enriched nanoparticles significantly increases their uptake by dendritic cells. Aminophospholipids are common components of the cell membrane bilayer; phosphatdylethanolamine is mostly present in the inner leaflet of the cell membrane [5] and is actively translocated to the cytosolic surface by ATP-dependent mechanisms [6]. Although the migration capability of DC labeled with DPPE-enriched fluorine nanoparticles is diminished when compared to non-enriched nanoparticles given at the same PFCE labeling concentration, lower concentrations of DPPE-enriched fluorine nanoparticles still resulted in a higher ^{19}F signal per million cells and minimal decrease in migration. Our results suggest that the incorporation of DPPE in the composition of PFCE nanoparticles could help to offset the sensitivity constraints of ^{19}F MRI with the ultimate goal to facilitate tracking of even small numbers of DCs in vivo.

References: 1. Ahrens ET et al. In vivo imaging platform for tracking immunotherapeutic cells. Nat. Biotechnol 2005;23: 983-987. **2.** Waiczies H et al. Perfluorocarbon particle size influences magnetic resonance signal and immunological properties of dendritic cells. PLoS One 2011;6: e21981. **3.** Waizies H et al. Visualizing brain inflammation with a shingled-leg radio-frequency head probe for 19F/1H MRI. Sci Rep 2013;3: 1280. **4.** Bendix I et al. MAPK3 deficiency drives autoimmunity via DC arming. Eur. J. Immunol. 2010;40: 1486-1495. **5.** Zachowski A Phospholipids in animal eukaryotic membranes: transverse asymmetry and movement. Biochem J 1993;294 (Pt 1): 1-14. **6.** Müller K et al. Protein-dependent translocation of aminophospholipids and asymmetric transbilayer distribution of phospholipids in the plasma membrane of ram sperm cells Biochemistry 1994;33: 9968-9974.