Multicolor 19F-MRI for *in vivo* Imaging of immune cells activity in a model of Multiple Sclerosis

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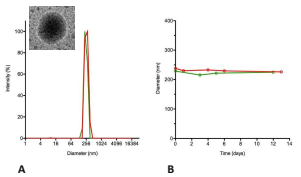
Introduction

The present work is focused on the development of a MRI protocol to track infiltrating immune cells in order to image inflammation-related diseases and thus to monitor disease progression. Among all the currently available imaging modalities, Fluorine nanoparticles (19F-NPs) have been shown to be a promising tool since 19F-signal is quantitative and highly specific thanks to the absence of biological background.

Different studies demonstrate the ability of MRI with **19F-NPs to highlight inflammation or to perform cell tracking**. We investigated the potential use of a new super-fluorinated molecule **PERFECTA** which considerably improved MR sensitivity. **PERFECTA** could be used simultaneously with the standard **PFCE nanoparticles** since both compounds have a single peak at two distinct chemical shift which able multi-spectral imaging. Indeed, both 19F-NPs could be used to label two distinct targets or cell populations and could be detected together in vivo. In the present work, we explored the suitability of 19F-MRI to follow in vivo the dynamics of immune-cells infiltration in an EAE model using a multi-color MR imaging tool with two 19F-NPs (PFCE and PERFECTA).

Methods

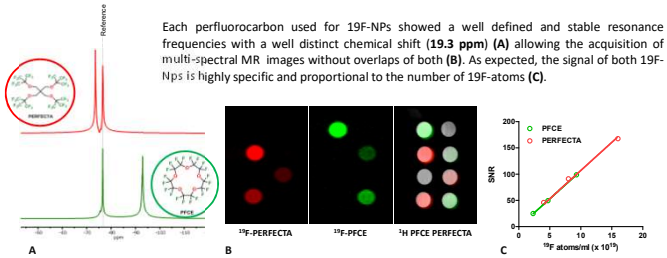
19F-Nanoparticles synthesis and characterization



Fluorescent 19F-NPs were produced emulsifying two different perfluorocarbons (PFCE and PERFECTA) with a non-ionic surfactant by direct tip sonication. All 19F-NPs were characterized for: size, stability (DLS), MR properties and also fluorescence profile.

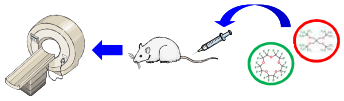
A. Nanoparticle size distribution measured by Dynamic Light Scattering in emulsions diluted in water for each PFC; **B.** The size of both 19F-NPs were stable over weeks.

Fluorine (19F) multicolor MRI



Each perfluorocarbon used for 19F-NPs showed a well defined and stable resonance frequencies with a well distinct chemical shift (**19.3 ppm**) **(A)** allowing the acquisition of multi-spectral MR images without overlaps of both **(B)**. As expected, the signal of both 19F-Nps is highly specific and proportional to the number of 19F-atoms **(C)**.

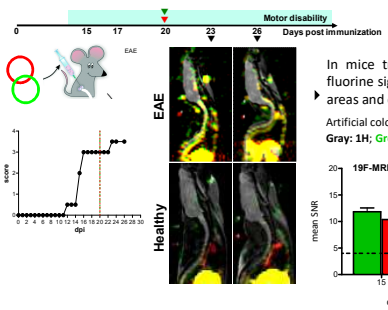
In vivo 19F-MRI method



Using a 7Tesla MRI preclinical scanner, longitudinal imaging experiments were performed in vivo in a model of multiple sclerosis (EAE mice, n=15) and healthy controls (n=9). For EAE induction, C57BL/6 mice (female, 6-8 weeks old) were immunized with a MOG peptide and received two dose of pertussis toxin at 0 and 2 days post immunization (dpi). The clinical score and body weight of all mice were daily recorded using a standardized scale for motor disability evaluation. For each MRI scan, the signal of each 19F-Nps was quantified in different areas of CNS (Brain and Spinal Cord), lymphoid organs (lymph nodes, thymus and bone marrow) and liver.

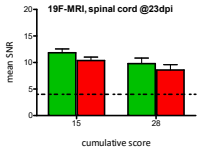
Results

Both 19F-NPs have the same performance *in vivo*



In mice treated simultaneously with both 19F-NPs, fluorine signal was similarly distributed over the same areas and co-localize with the same intensity .

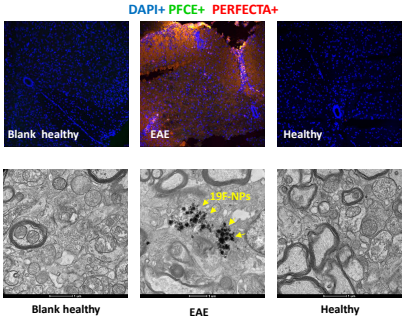
Artificial colors were assigned to each acquired MR image:
Gray: 1H; **Green: PFCE** and **Red: PERFECTA**



Mean signal to noise ratio (SNR) measured in the spinal cord from EAE mice with different disease severity

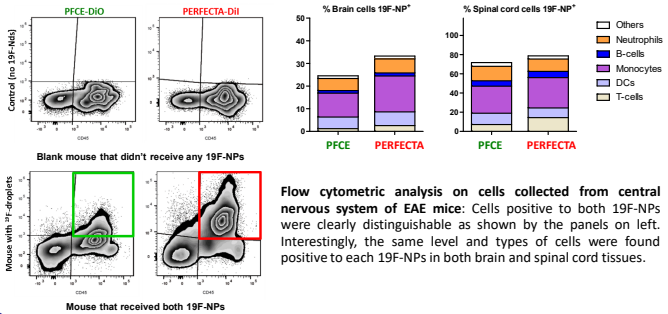
Ex-vivo characterization of Fluorine uptake in the spinal cord

Cytofluorimetric, immunofluorescence microscopy and transmission electron microscopy (TEM) analysis were done on different organs to further validate and characterize 19F-labeled cells. As control, healthy and EAE mice not treated with 19F-NPs were used (blanks).



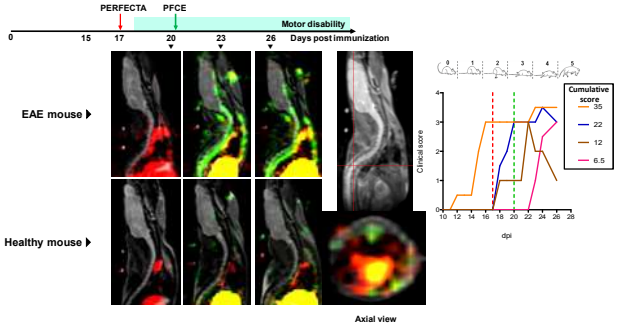
Immunofluorescence microscopy on spinal cords: both 19F-NPs co-localize (yellow) within the spinal cord of EAE mice while, no 19F-NPs could be found in the healthy animals.

Transmission electron microscopy on spinal cords: 19F-NPs were clearly found (black spots) in EAE mice compared to treated and untreated healthy mice.

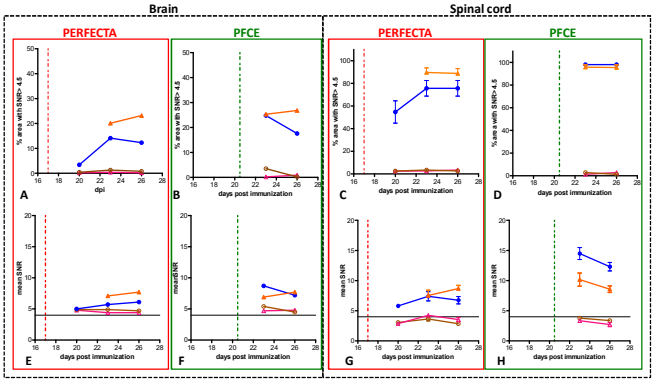


Flow cytometric analysis on cells collected from central nervous system of EAE mice: Cells positive to both 19F-NPs were clearly distinguishable as shown by the panels on left. Interestingly, the same level and types of cells were found positive to each 19F-NPs in both brain and spinal cord tissues.

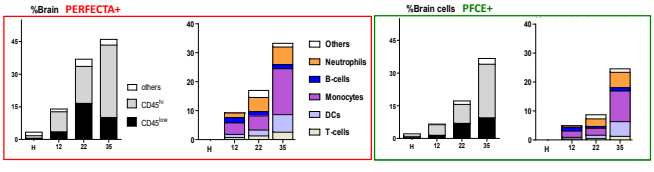
19F-Multicolor MRI to track different intensity of immune cells activation in EAE model



Quantification of Fluorine uptake in EAE mice



Fluorine signal quantification on EAE central nervous system from 19F-MRI: 19F-NPs uptake was expressed as the % of area with a Signal to Noise Ratio (SNR) > 4.5 (detection treshold)**(A-D)** and its mean SNR **(E-H)**. An important uptake was found in the spinal cord **(C-H)** and in the brain **(A-F)** of EAE mice with a severe disease (high cumulative score). In particular, 19F-NPs was high with the contrast agent administrated after EAE onset (PFCE).



Flow cytometric analysis on EAE brains: The percentage of cells positive to the fluorescent-19F-NPs increased with EAE severity, which is associated with increased number of leukocytes infiltration into the CNS. Interestingly, cells positives to each 19F-NPs were mainly monocytes, neutrophils and dendritic cells

Conclusions

Our results demonstrates the potentiality of multicolor 19F-MRI to track immune cells activation during different phases of pathological progression in the complex context of MS. The proposed 19F-NPs could be extended also to therapeutic cells to monitor their efficacy and localization over time. Thus **multicolor** 19F-MRI could be of great interest to label both therapeutic cells and the circulating immune cells with the aim to monitor the effects on inflammation.

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