# Efficient labeling of dendritic cells with a clinical applicable perfluorpolyether compound for quantitative 19F MRI tracking in cancer patients

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## Introduction

Dendritic cells (DCs) are the professional antigen-presenting cells of the immune system. Following infection or inflammation they undergo a complex process of maturation, and migrate to lymph nodes where they present antigen to T cells. Their decisive role in inducing immunity formed the rationale for DC immunotherapy: DCs loaded with tumor antigens are injected into cancer patients to stimulate T cells to eradicate tumors. Effective immune responses and favorable clinical outcomes have indeed been observed, but only in a minority of patients. To obtain a better understanding of the migratory mechanisms exploited by DCs to improve vaccine efficacy we developed novel MRI-based imaging techniques to trace and quantify DCs in vivo. We exploited a clinical applicable $^{19}$F MRI compound as a potential label for DCs.

$^{1}$H and $^{19}$F images of labeled cells and surrounding tissue provide information on anatomical localization and quantification respectively [1]. The sensitivity of this compound is determined in vitro and compared with typical SPIO based contrast agents in phantoms as well as in tissues.

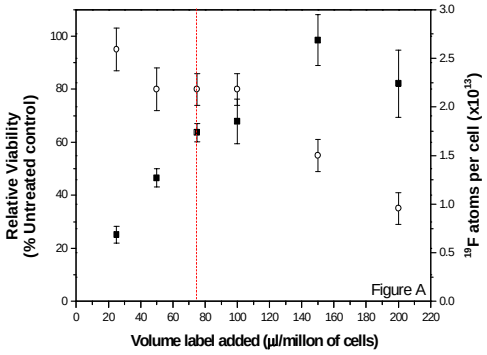
## Methods

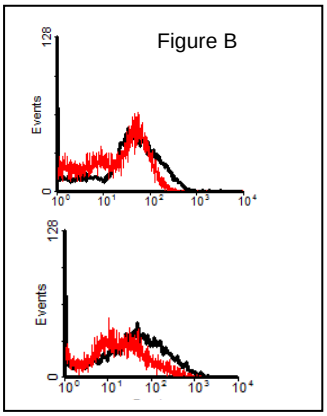
DCs were generated from peripheral blood mononuclear cells and matured as previously described [2]. For $^{19}$F-labeling, a PFPE based compound (Celsense Inc., Pittsburgh PA) was added at day 3. After maturation, cells were harvested, and viability determined by tryphan blue exclusion. Maturation marker expression by labeled DC was determined by flow cytometry. MRS at 282.4 MHz was used to determine $^{19}$F content per cell with a calibrated reference (triflouracetic acid). $^{1}$H and $^{19}$F images were performed on a 7T horizontal bore MR-system with a 10 mm diameter $^{1}$H/$^{19}$F surface coil. For MRI, a variable number of labeled DCs were embedded in gelatin. Proton images were acquired with a 2DFT spin-echo sequence with TR/TE=1000/22 ms and 0.125×0.125×1 mm$^3$ resolution. $^{19}$F spin density weighted images were measured using spin-echo sequence with TR/TE=1300/4 ms and 1×1×2 mm$^3$ voxels.

## Results

The labeling protocol we developed results in about $2x10^{13}$±$9x10^{11}$ $^{19}$F atoms per cell, with a viability of 80±6% (Figure A).

We found minimal effects of the label on the DCs. In particular, maturation, mRNA uptake via electroporation (Figure B), and both allogeneic as well as antigen-specific activation of T cell by the DCs were not affected by the $^{19}$F label. Proton and $^{19}$F Fluorine images of a fixed amount of embedded cells and a reference sample are shown in the inset of Fig. C. The cells/reference signal intensity ratio dependence with the embedded cell density is plotted in Fig. C. A clear linear relationship is obtained, which allows for quantification.



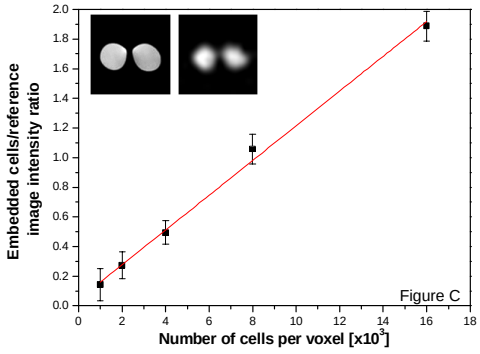




***Fig. A**: Cytotoxicity (open circles, left axis) and label uptake by the DCs (full squares, right axis) dependence with the volume of label added to the cells. In view of both results, the optimum volume of label to be added can be determined. **Fig. B**: Expression of maturation marker CD86 (top) and mRNA (gp100 tumor antigen) uptake (bottom) via FACS. There are no remarkable differences between labeled (red) and unlabelled cells (black). **Fig. C**: $^{19}$F image intensity ratio of embedded cells and a reference for different number of cells per voxel. $^{1}$H (left) and $^{19}$F (right) images of 8000 cells/voxel embedded cells (left tube) and a reference (right tube) are shown in the inset of this figure.*

## Discussion and Conclusions

A new PFPE based compound was tested for DC tracking with $^{19}$F MR imaging. The compound demonstrated three major advantages: efficient uptake by the cells, minimal effect on cell viability at relatively high concentrations and a sufficient number of $^{19}$F atoms to image a minimum cell density of 500 cells/mm$^3$ (comparable to sensitivity with SPIO) [2]. The sensitivity of this compound is not expected to change substantially in vivo. This constitutes a key advantage when compared with typical proton contrast agents. In conclusion, we believe that the PFPE-based compound described is very useful for labeling ex vivo generated cells which maintain the capacity to exert their function in vivo.

## References

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