Simultaneous and quantitative tracking of distinct cell populations using 19F MRI

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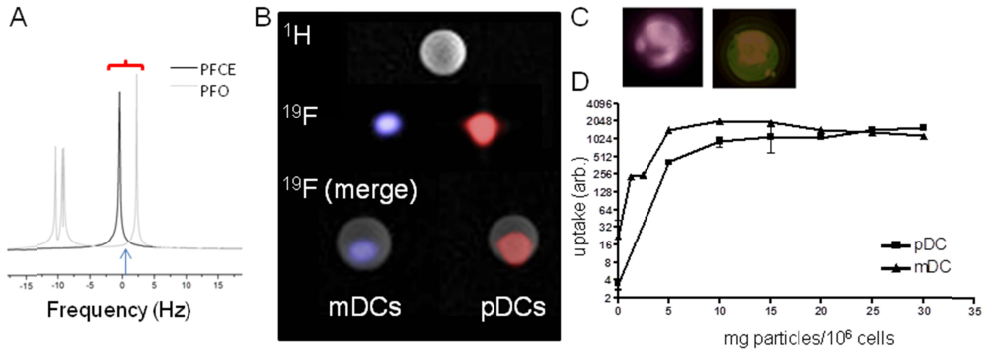
Introduction

The surge of interest in cellular therapeutics demands a technique for the localization and quantification of these cells after transfer to the subject. However, imaging using typical iron-based contrast agents results in dark spots in images, which can obscure anatomic detail, and quantification is difficult. Hence, "hot-spot" imaging is coming to the fore, primarily using ^{19}F labels [1]. ^{19}F MRI allows direct quantification of label content, and therefore cell numbers, from the image data. Here we exploit the technique further to show the simultaneous tracking of two distinct dendritic cell (DC) populations using different ^{19}F labels. The labels we use here consist of polymer-encapsulated perfluorocarbons (PFCs) [2]. The use of distinct ^{19}F resonances has been proposed previously by us and other groups [3]. However, here we image both labeled cell populations simultaneously using a sequence with a sufficiently broad receiving bandwidth (Fig A; red brace indicates receiving bandwidth and arrow the transmitter frequency). We also show that the technique is quantitative for both compounds over a large range of concentrations. The particles are synthesized using components approved for clinical use, where possible.

Methods

Primary human DCs were cultured, as per standard protocols for DC vaccination trials [4]. Here, we used plasmacytoid and myeloid DCs (pDCs and mDCs). Cells were incubated with the ^{19}F label, washed and studied further. Nanoparticles were formulated with PFC [2], here we used perfluoro-15-crown-ether (PFCE) and perfluorooctane (PFO) and distinct fluorescent dyes. 0.5 million labeled mDCs and 1 million pDCs were imaged here. MRI was done on a 7T horizontal scanner, using a modified gradient echo sequence. False color ^{19}F images (TR/TE=200/2.82ms, 25° flip angle, 1.88×0.94×2mm voxels, 256 averages, 12min; bandwidth of 240 Hz/pixel) are overlaid on corresponding grayscale ^{1}H images (TR/TE=1200/14ms, 0.12×0.6×2mm voxels, 1 average). Post-processing to correct for the frequency shift was done using the known frequency of the label compounds and the bandwidth per pixel in the images.

Results and Discussion



The imaging sequence allows for simultaneous acquisition of both signals (B; ^{19}F false color with PFO in red and PFCE in blue, where the lower panel shows the final image overlaid on the ^{1}H grayscale image). Note that this is not chemical shift imaging, but a modified gradient echo sequence. Thus, the imaging time is much reduced. The image shows the processed ^{19}F image obtained. Furthermore, we found that the received signal is quantitative for both frequencies, over a large range of concentrations (not shown). The addition of fluorescent dyes allows fluorescent imaging (C; labeled mDCs on left and pDCs on right). The use of in vivo or ex vivo fluorescence imaging can be used to confirm the ^{19}F-based cell localisation. This can also be done by adjusting the ^{19}F excitation and acquisition parameters to exclusively detect a single cell type, when time permits. We also found that there was no effect on the primary human DC populations labeled, in terms of maturation, ability to active lymphocytes and migration. Importantly, these DCs took up sufficient quantities of label even though they are not phagocytic, and the label was contained within the cells (not shown). The label uptake shows a typical saturation curve for both cell types (D). In summary, using this technique it is possible to measure the migration of two cell populations quantitatively and simultaneously.

Conclusion

The ability to quantitatively image two compounds simultaneously allows for much shorter imaging times than with chemical shift imaging. This imaging technique makes the tracking of multiple distinct cell populations in vivo feasible.

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References

[1] Srinivas M, Heerschap A, Ahrens ET, Figdor CG, de Vries IJ. (19)F MRI for quantitative in vivo cell tracking. Trends Biotechnol. 2010 Jul;28(7):363-70. Review.
[2] Srinivas M, Cruz LJ, Bonetto F, Heerschap A, Figdor CG, de Vries IJ. Customizable, multi-functional fluorocarbon nanoparticles for quantitative in vivo imaging using 19F MRI and optical imaging. Biomaterials. 2010 Sep;31(27):7070-7.
[3] Partlow KC, Chen J, Brant JA, Neubauer AM, Meyerrose TE, Creer MH, Nolta JA, Caruthers SD, Lanza GM, Wickline SA. 19F magnetic resonance imaging for stem/progenitor cell tracking with multiple unique perfluorocarbon nanobeacons. FASEB J. 2007 Jun;21(8):1647-54.
[4] de Vries IJ, Lesterhuis WJ, Barentsz JO, Verdijk P, van Krieken JH, Boerman OC, Oyen WJ, Bonenkamp JJ, Boezeman JB, Adema GJ, Bulte JW, Scheenen TW, Punt CJ, Heerschap A, Figdor CG. Magnetic resonance tracking of dendritic cells in melanoma patients for monitoring of cellular therapy. Nat Biotechnol. 2005;23(11):1407-13.