Monitoring the Uptake of ^{19}F Nanoparticles and In Vivo Migration of Dendritic Cells using Magnetic Resonance

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Introduction

^{19}F magnetic resonance imaging (MRI) offers a number of advantages to track cells *in vivo*. These include improved signal selectivity and a possibility to overlay ^{19}F-labeled cells with anatomic ^{1}H scans. Indeed ^{19}F/^{1}H MRI should prove to be an important adjunct for the delivery of cellular therapies in clinical applications. This work investigates the uptake of nanoparticles containing ^{19}F perfluoro-15- crown-5 ether in dendritic cells and the impact of these nanoparticles on cell function. ^{19}F MR spectroscopy and electron microscopy was performed to monitor ^{19}F uptake and ^{19}F/^{1}H MRI was employed to track DC migration *in vivo*.

Methods

Dendritic cells (DC) from C57BL/6 mice were labeled with an emulsion containing perfluoro-15-crown-5 ether (Fluorochem, Derbyshire, UK) that was prepared as previously described.[1] Median diameter of the particles in the emulsion was determined by dynamic light scattering (Zetasizer Nano ZS, Malvern Instruments, Worcestershire, UK). After ^{19}F-labeling in 100 mm Petri dishes, DC were thoroughly washed and fixed in 2% PFA. The ^{19}F signal intensity in DC was measured 3 h, 24 h and 48 h after labeling using a 3T MRI system (MedSpec 30/100 Bruker, Ettlingen, Germany). A ^{19}F tuned, 5-turn loop coil (5 mm inner diameter to hold NMR-tubes, 15 mm long) customized for the 3T scanner was used for signal transmission and reception. A 90° block pulse with 10 kHz bandwidth was used for excitation. The intracellular uptake of the nanoparticles in DC was visualized by electron microscopy: cell pellets were fixed (24 h) using 2 % glutaraldehyde, postfixed (2 h) with 1 % osmium tetroxide, dehydrated in ethanol and embedded in Poly/Bed 812 (Polysciences, Eppelheim, Germany). Semithin sections of the pellets were stained with toluidine blue. Ultrathin sections were stained with uranyl acetate and lead citrate. ^{19}F-labeled DC (10^7) were administered intradermally into the hind limb of C57BL/6 mice. To follow the migration of the ^{19}F-labeled DC *in vivo* a Biospec 94/20-USR (Bruker, Ettlingen, Germany) animal scanner was used. A ^{19}F surface coil tuned to 376 MHz was customized for measurements on the 9.4T scanner and assembled inside a ^{1}H volume birdcage resonator.

Results

The ^{19}F nanoparticles were efficiently taken up by DC. ^{19}F MR spectroscopy revealed that ^{19}F signal is directly dependent on the loading time and concentration of perfluoro-15-crown-5-ether as demonstrated in Fig. 1. ^{19}F particles were taken up by DC already 3 h after application as shown in Fig. 1. Electron microscopy imaging revealed the ^{19}F nanoparticles as clusters of bright smooth spheroids (Fig. 2) with an average size of 660 nm ± 280 nm (n = 150) and as shown in ultrathin sections, these clusters were commonly enclosed within a membrane-like surface and an amorphous grey compartment (Fig. 2 d), a phenomenon that has been observed in cells loaded with polyisoprene nanoparticles.[2] According to dynamic light scattering the average of the median particle size was calculated to be 245 nm (± 1.97 nm) and this increased to 560 nm (± 6.36 nm) following autoclaving. Interestingly, uptake of the particles in DC was directly dependent on particle size as shown by an increased ^{19}F signal intensity in cells labeled with the autoclaved (larger particle size) emulsion (Fig. 3). The latter particles were also tolerated by DC as shown by immunological tests measuring viability, apoptosis rate and function of these cells following labeling. Furthermore ^{19}F-labeled DC were capable of reaching draining popliteal lymph nodes following antigen loading and intradermal application in the hind foot pad as illustrated in Fig. 4.

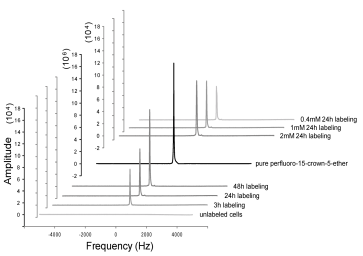

Fig. 1: Monitoring ^{19}F nanoparticle uptake by DC using ^{19}F MR spectroscopy. The ^{19}F signal in fixed DC pellets is directly dependent on the loading time and concentration of perfluoro-15-crown-5-ether.

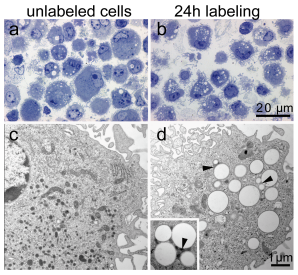

Fig. 2: Monitoring ^{19}F nanoparticle uptake using electron microscopy of semithin sections (a, b) and ultrathin sections (c,d) of DC pellets. In ^{19}F-labeled DC (d) most vesicle clusters are enclosed in a membrane-like surface and an amorphous grey compartment (arrowhead).

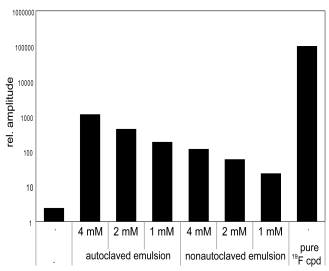

Fig. 3: An increase in particle size (autoclaved emulsion) results in a higher ^{19}F signal intensity in DC as determined by ^{19}F MR spectroscopy.

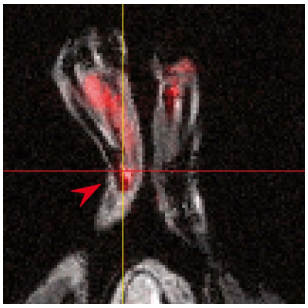

Fig. 4: ^{19}F MR images superimposed with ^{1}H scans obtained 24 h after injection of labeled DC. Red arrow indicates ^{19}F-labeled cells (red) reaching the popliteal lymph nodes.

Conclusions

Nanoparticles containing the ^{19}F compound perfluoro-15-crown-5 ether were rapidly and efficiently taken up by DC; the ^{19}F signal intensity in these cells being directly related to the particle size. No toxicological effects were observed following application of the ^{19}F nanoparticles in DC and the cellular function – particularly *in vivo* migration – was maintained. ^{19}F MRI facilitated monitoring of DC migration into draining popliteal lymph nodes, which holds the potential to pave the way towards clinical applications, particularly for the delivery of cellular therapies in the field of cancer, infection, autoimmunity or transplantation.

References

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2. Dass K, Landfester K, Walther P. Cellular Uptake of Polymer Nanoparticles Imaged by Electron Microscopy Based on High-Pressure Freezing. Microscopy and Microanalysis 2007; 13:220-221.