In vitro / In vivo MRI visualization of PFC / VSOP double-labeled mesenchymal stem cells

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Introduction:

Stem cell based therapies are a promising approach to face multiple medical questions. Therefore, non-invasive monitoring of stem cells *in-vivo* is an important issue for therapy development and evaluation. Superparamagnetic iron oxide (SPIO) particles can be used as MRI markers for this purpose [1,2]. T_2* weighted MR images are very sensitive to SPIOs which cause hypointensities in their surrounding allowing to detect small numbers of cells in short measurement times with highly resolved ^{1}H MRI. The "dark spots" can also be induced by other causes, e.g. blood clots. Thus, monitoring of cells labeled with SPIOs is difficult especially in heterogeneous tissue. Recently, perfluorocarbon (PFC) particles have shown their potential in being a non-ambiguous MRI marker for detection of cells *in-vitro* and *in-vivo* [3,4,5]. Due to the relatively weak fluorine signal from the PFCs, ^{19}F-MRI has limits to the number of detectable cells and resolution of ^{19}F images in short measurement time. In order to combine the advantages of both methods we present a study to monitor cells double-labeled with SPIO and PFCs *in-vitro* and *in-vivo* using ^{1}H/^{19}F-MRI.

Materials and Methods:

Cells from the human mesenchymal stem cell line hTERT were double-labeled. First, cells were labeled with perfluoro-15-crown-5-ether (PF15C) emulsions using electroporation. Subsequently, very small iron oxide particles (VSOPs®) were added to the medium of PF15C labeled cells and absorbed by the cells, resulting in a double-label of the cells. ^{1}H/^{19}F-MRI was preformed using a 7 T Bruker Biospec with a homebuilt, double-resonant birdcage-coil. To obtain highly resolved ^{1}H-MR images gradient echo sequences were used. Subsequently, lower resolved ^{19}F Turbo-Spin-Echo experiments were performed to identify the location of the double-labeled cells. For *in-vitro* experiments quantification of the intracellular PF15C content was performed using ^{19}F-MRS and additionally the intracellular iron quantity was measured with mass-spectroscopy. For *in-vivo* experiments a CD-1 nude mouse was narcotized using Avertin, eventually double-labeled cells were injected i.m..

Results:

The double-labeled cells can be easily detected *in-vitro* using both ^{1}H- and ^{19}F-MRI (c.f. Fig.1). ^{19}F-MRS and mass-spectroscopy enable the quantification of the intracellular marker content being PF15C particles and VSOPs. *In-vivo* double-labeled cells cannot be identified easily using the ^{1}H images only (c.f. Fig.2 A/C). Subsequently taken ^{19}F images allow the non-ambigous detection of double-labeled cells *in-vivo* (c.f. Fig.2 B/D). Due to the dephasing effect of the VSOP particles the 19F signal is attenuated (c.f. Fig. 1B).

Discussion and Conclusion:

Highly resolved ^{1}H-MR images and lower resolved ^{19}F-MR images were obtained in measurement times lasting only a few minutes. Labeled cells appear as dark spots or regions in the ^{1}H image and can be distinctly identified using ^{19}F-MRI. Double-labeling thus allows to non-ambiguously monitor labeled cells with high resolution and in combination with ^{19}F-MRS may lead to quantitative cell imaging in the future.

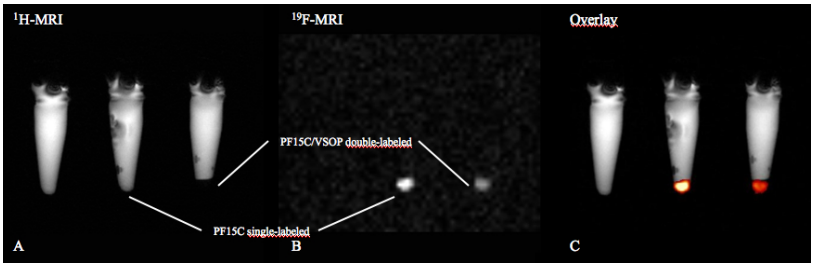

Figure 1: (A) ^{1}H MR-Image of control (left), single (middle) and double-labeled cells (right). The caps contained 2x 10^6 cells each. (B) Corresponding ^{19}F MR-Image. (C) Overlay of A and B.

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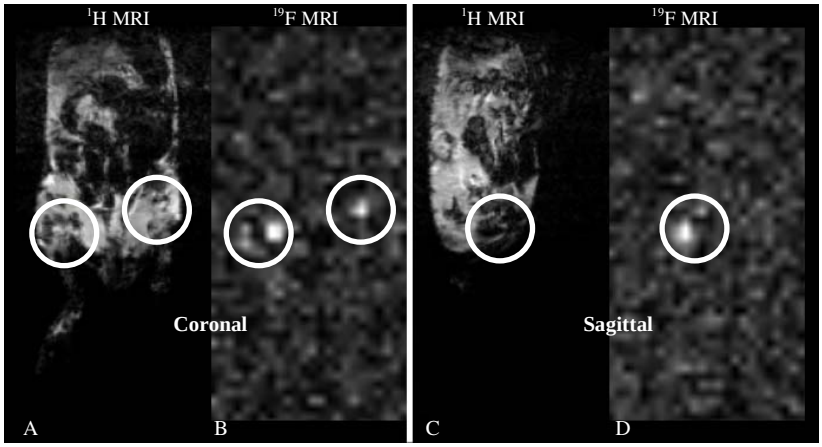

Figure 2: (A) Coronal ^{1}H MR-Image of the mouse. (B) Corresponding ^{19}F MR-Image. (C) Sagittal ^{1}H MR-Image of the mouse. (D) Corresponding ^{19}F MR-Image. Approximately 2.5 x 10^6 double-labeled cells were injected in the right thigh of the mouse and 1.25 x 10^6 in the left thigh. The ^{19}F images provide sufficient signal to identify the double-labeled cells.