Multi-color ^{19}F CSI: Simultaneous detection of differently labeled cells in vivo

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Introduction

Perfluorocarbon (PFC) emulsions have been used for cell tracking studies [1, 2]; furthermore, Flögel et al. recently showed it is possible to visualize inflammation in myocardial infarction and cerebral photothrombosis (PT) mouse models using ^{19}F MRI [3]. In contrast to conventional ^{1}H contrast agents, ^{19}F markers provide unambiguous signal *in vivo* due to the low natural abundance of fluorine in living organisms. A further advantage of ^{19}F molecular MRI is that fluorine markers possess a unique spectral signal. Therefore, chemical shift imaging (CSI) methods can be used to distinguish between cells labeled with different fluorine markers.
It was previously shown that macrophages labeled with different PFC compounds can be distinguished *in vitro* with a single multi-color ^{19}F CSI experiment [4]. This pilot study demonstrates the feasibility of distinguishing between differently labeled cells *in vivo* in a PT mouse model. This is possible by using fast and specific multi-color CSI methods.

Materials and Methods

To validate the method *ex vivo,* PFC emulsion phantoms were measured. T_2 weighted ^{1}H Turbo SpinEcho (TSE) reference scans were performed (TR/TE=5000/10ms, Turbofactor=8, FOV=30x30mm, Matrix=300x300, slices=16, SI=1mm, NA=1). Subsequently, ^{19}F CSI data was acquired (TR=13.6ms, Tacq=10.2ms, FOV=30x30mm, Spectral points=512, Matrix=48x48, slices=1, SI=2mm, NA=1).
Regarding *in vivo* experiments, focal cerebral lesions were induced in a C57/BL6 mouse by photothrombosis as described previously [5, 6]. Ten days after right cortical infarction, another PT lesion was induced on the left hemisphere. The mouse received different PFC compounds intravenously: VS 580H after the first surgery and VS1000H after left-side ischemia (Celsense Inc., Pittsburgh, PA, USA). MRI was performed 3-, 13-, and 21-days after the induction of the right cortical PT. Afterwards, the animal was sacrificed and the brain was removed, fixed in 4% paraformaldehyde and embedded in paraffin. Coronal sections were cut through the infarctions and HE-staining was performed. The same imaging parameters were used as in *ex vivo* measurements (^{19}F CSI: NA=115). All measurements were performed on a Bruker BioSpec 7 T (Bruker Biospin GmbH, Rheinstetten, Germany) using a customized ^{1}H/^{19}F double-resonant birdcage coil.
For postprocessing, ^{1}H TSE and ^{19}F CSI data were zerofilled to a matrix size of 512x512. Additionally, CSI data was zerofilled to 1024 spectral points. The location of the different compounds was determined by calculating ^{19}F compound (frequency) selective images from the B_0 shift corrected and zerofilled ^{19}F CSI data. To further validated the method, similar *in vivo* and *in situ* experiments were performed on additional mice (n=2, data not shown).

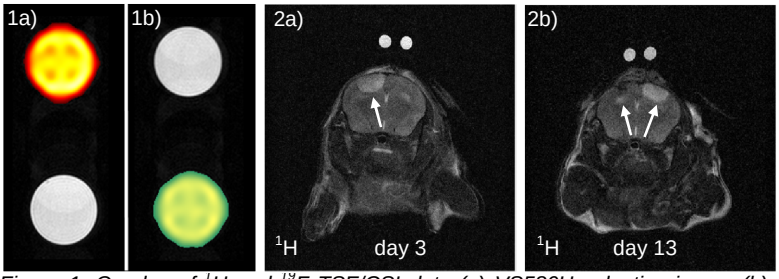

Figure 1: Overlay of ^{1}H and ^{19}F TSE/CSI data (a) VS580H selective image (b) VS1000H selective image. Figure 2: ^{1}H TSE images measured on different days after surgery.

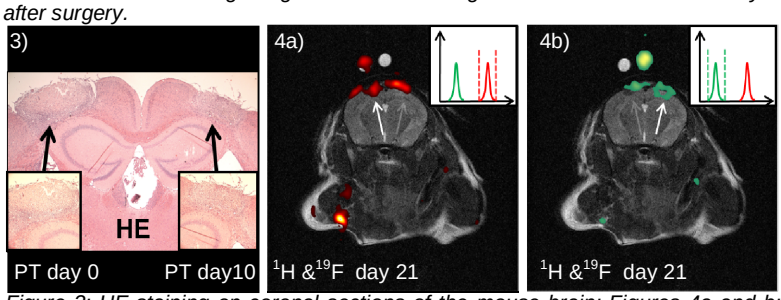

Figure 3: HE-staining on coronal sections of the mouse brain; Figures 4a and b: Overlay of ^{1}H and ^{19}F TSE/CSI datasets (a) Frequency selective image of the VS580H emulsion (b) Frequency selective image of the VS1000H emulsion.

Results

Figure 1 illustrates the *in vitro* results. Figure 1a shows the tube containing VS 580H and Figure 1b shows the VS1000H-tube. Both frequency selective images were calculated out of the same ^{19}F CSI data.
In Figures 2 a-b, ^{1}H TSE images are shown taken 3- (fig. 2a) and 13- (fig. 2b) days after induction of the first cortical infarction. Note that the first PT lesion (arrows) appears hyperintense on ^{1}H TSE images early after induction (fig. 2a) and hypointense with lesion maturation (fig. 2b). In contrast the second delayed PT lesion still appears hyperintense (fig. 2b).
Figure 3 shows HE-staining on coronal brain sections with cortical infarctions on both sides of the cortex.
Figure 4a shows the overlay of the ^{1}H image with the B_0 shift corrected, zerofilled and summed up ^{19}F CSI data, which contains the peak signal of the VS580H emulsion. The ^{19}F signal (VS580H) is easily recognizable within the right cortical infarction.
Figure 4b illustrates the overlay of the VS1000H peak signal and the ^{1}H reference image. The sketch in the upper right corner of Figures 4a and b demonstrates the different spectral range chosen for calculating the frequency selective images. In both cases, the ^{19}F signal reflects labeled cells, which are supposedly macrophages infiltrating the ischemic lesion.^{19}F signal was also observed supracranially at the location of skin incision.

Conclusion

Multi-color ^{19}F CSI allows distinction between cells labeled with different PFC compounds *in vivo*. Previous studies showed that PFCs can be taken up by macrophages, B-cells and dendritic cells [1, 2, 3], thus leaving the possibility of *in vivo* tracking and quantification of different immune cells within a single multi-color ^{19}F CSI experiment.

References

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