Imaging of SPIO Labelled Endothelial Networks at 3, 7 and 11.7 Tesla

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Introduction

Endothelial progenitor cells (EPC) are promising candidates for novel stem cell transplantation strategies to treat cardiovascular diseases. Within this study formations of vascular network structures with super paramagnetic iron oxide (SPIO) loaded EPCs were induced, which is an in vitro correlate of the vasculogenesis process [1]. The resulting endothelial network showed to be an optimal candidate to optimize labelling strategies as well as protocols for single cell detection. High resolution (55µm) images at 3, 7 and 11.7 Tesla systems were investigated and the impact of different field strength and gradient performance on the image quality was examined.

Materials and Methods

Cell preparation: Cinical scale EPC propagation was done in a novel animal serum free system. Cell labelling was performed without the aid of transfection agents within 24h in 280µg/ml Fe Resovist (Schering, Germany) solution to prevent cell death or dysfunction. Single cell SPIO uptake was visualized by using Prussian Blue staining and quantified by flow cytometry. Sub-cellular SPIO distribution was investigated by transmission electron microscopy. Cell cultures were seeded in 24 and 48 well plates containing extracellular matrix solution for the formation of endothelial networks. Resulting networks were washed and the wells were filled with Gd-DTPA (0.5mmol/l) doped agarose-gel in order to reduce T1 of the cell environment.

Pulse sequences: The scans were performed on a 3T Tim Trio (Siemens Medical, Germany) clinical system with 38mT/m gradient strength and a 18mm surface coil (Rapid Biomedical, Germany), a 7T (Bruker BioSpin, Germany) system with maximum gradient performance of 1500mT/m using a 25mm birdcage coil and a 11.7T (Bruker) system with a 3000mT/m gradient and a saddle coil of 10mm inner diameter.

After 2nd order shimming, 3D FLASH images were acquired with following parameters: 3T: 384x384 matrix, resolution 57x57x62 µm, TR/TE 200/14 ms, FA 20°, NSA 12; 7T: 384x384 matrix, resolution 57x57x78 µm, TR/TE 200/3.2 ms, FA 30°, NSA 4; 11.7T: 256x256 matrix, resolution 55x55x55 µm, TR/TE 200/2.5 ms, FA 30°, NSA 8.

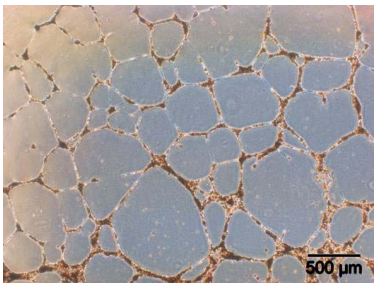

Fig. 1: Microscopic image of SPIO labelled endothelial network.

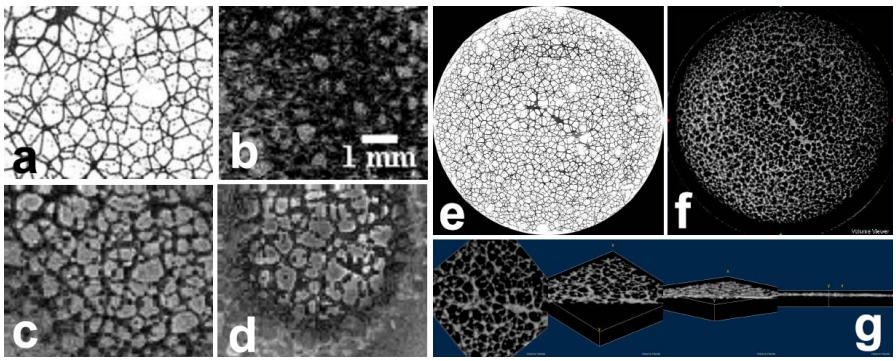

Fig. 2: Microscopic (a), 3T (b), 7T (c) and 11.7T (d) image of the cell network. Images taken at 7T benefit from short echo times and high SNR and allow a one-to-one registration of every single structure compared with the microscopic counterpart. A minimum intensity projection (f) calculated from the 7T 3D dataset presents a 3D view (g) of the microscopic cell cluster (e).

Results and Discussion

Microscopic images (**Fig. 1**) of the cell networks show the equally distributed SPIO particles as black dots within the cytoplasm. Even the structures interconnecting the cells were loaded with iron oxides and therefore yield to good contrast at the GRE imaging method. The uniform distances between the cells proved to be ideal to allocate single cells and allowed to optimize SPIO cell load and pulse sequences.

For images taken with 3T (**Fig. 2b**), only cells with distances greater than 200µm could be detected separately. The used conventional clinical gradient system restricted the echo time TE to 14ms. Strong de-phasing of voxels in close proximity to the cells caused a broadening of the structures. Nevertheless, cell structures were clearly visible and reducing the iron content per cell would improve the image quality.

7T MR images (**Fig. 2c**) showed a good signal-to-noise ratio and the used gradient system allowed echo times down to 3.2 ms. Shimming of the SPIO labelled structure at 7T was no remarkable problem. No signal variations due to off-resonant areas occurred. This allowed to calculate a minimum intensity projection through the whole imaging volume. The result, showed in figure **2e-g**, presented a three dimensional view of the cell cluster which might be used to study vessel wall growth in a more detailed manner.

Despite the superior SNR of the 11.7T system, the image quality strongly depended on the reduction of SPIO induced field-inhomogeneities. Off-resonant signal variations around the iron oxide labelled cells made 3D post-processing difficult.

In summary, SPIO labelled EPC networks are potentially good models to compare different systems, pulse sequences and cell detection strategies because their organized structure makes it easy to distinguish between single cells. Within this in-vitro study three different MR systems were compared. The 7T seemed to be the best candidate for single cell detection with GRE acquisitions because of its ability for short TEs and good shimming properties.

Acknowledgement

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Reference

[1] Wilhelm C. *et al*, *Biomaterials* 2007; 28:3797-3806.