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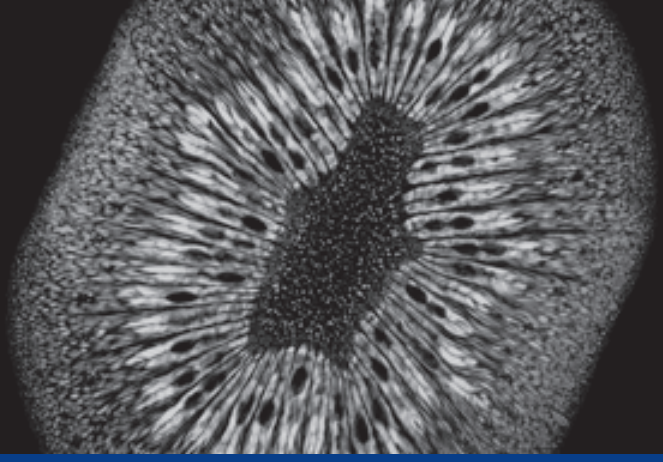
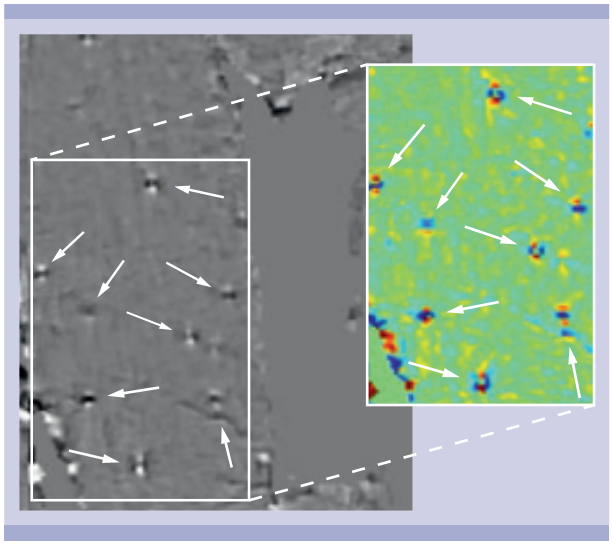


MRI tracking of SPIO-labeled bacteria



Phase-contrast MRI using FeraSpin™ R to track bacterial translocation in a mouse model of Graft-versus-Host Disease

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Introduction

Graft-versus-Host Disease (GvHD) is one of the most frequent complications following allogeneic tissue transplantation where the immune cells of the graft tissue attack the host's body cells, impairing their ability to function. Total body irradiation (TBI) is a procedure that may be applied prior to transplantation in order to suppress the recipient's immune system. However, TBI increases the risk of infection, particularly within the gastrointestinal tract. In this study, we show that it is possible to track bacterial infection after TBI *in vivo* using superparamagnetic iron oxide (SPIO) nanoparticle-labeled bacteria[1,2] and highly sensitive phase-contrast magnetic resonance imaging (MRI)[3,4].

Materials and methods

Escherichia coli (*E. coli*) labeling with FeraSpin R

Gram-negative *E. coli* bacteria were incubated at an iron concentration of 1 mM for 24 h with FeraSpin™ R (Viscover™, *nanoPET Pharma* GmbH, Germany), an MRI agent consisting of SPIO nanoparticles. The cell suspension was centrifuged and the supernatant was collected (Supernatant 1). The cell pellet was redispersed in PBS and centrifuged again. After removal of the supernatant (Supernatant 2) and redispersion of the pellet in PBS, the final SPIO-labeled bacterial cell suspension (SPIO$^+$*E. coli*) was used for injection in mice.

MRI procedure

To confirm labeling of *E. coli* cells with FeraSpin R, *in vitro* MRI was performed in a gel phantom. *In vivo* experiments were conducted in healthy BALB/c mice where 1 x 10^7 SPIO$^+$*E. coli* cells were injected into the rectum and lower colon of mice, which had either received 900 cGy TBI 48 h before (treated; n = 6), or which had not received treatment (control; n = 6). Scanning was performed on both animal groups before, 4 h and 24 h after injection of the SPIO$^+$*E. coli* using a 7 T BioSpec system equipped with a cryogenically-cooled quadrature resonator (Bruker, Ettlingen, Germany). The protocol consisted of a 3D FLASH sequence (T_R/T_E = 20/3.04 ms, resolution 50 × 52 × 52 µm^3, FA = 10°, bandwidth 55 kHz, acquisition time 9 min 12 s) and a 3D gradient echo (GE) flow-compensated sequence (T_R/T_E = 120/10 ms, FA = 22°, spatial resolution 40 × 40 × 250 µm^3, bandwidth 28 kHz, acquisition time 8 min 57 s). Both sequences were chosen to provide information about the migration of SPIO$^+$*E. coli* based on their R_2 relaxation rates and their magnetic susceptibility, respectively.

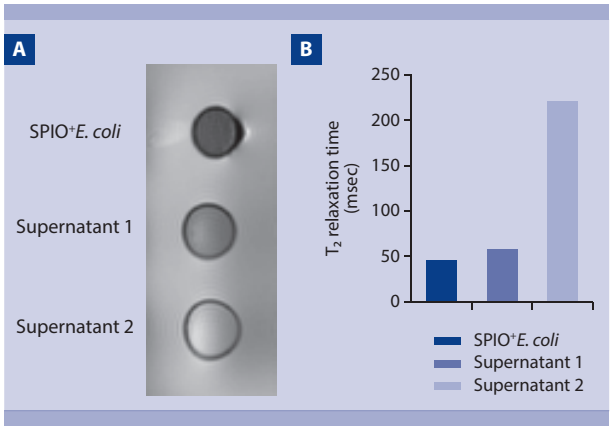

Figure 1: Verification of *E. coli* labeling with FeraSpin R.
A. *In vitro* MRI gel phantom of the first supernatant (Supernatant 1), the second supernatant (Supernatant 2) and the FeraSpin R-labeled bacteria (SPIO$^+$*E. coli*.) **B.** T_2 relaxation times showing successful bacterial labeling, through a significant shortening of the relaxation time of the SPIO$^+$*E coli*.

Data processing

GE flow-compensated images were processed offline with custom-made software developed in Matlab to reconstruct both magnitude and phase images. Local phase changes are visible due to the susceptibility difference between the SPIO$^+$*E. coli* and the surrounding intestinal tissue. This difference produces a local discontinuity in the magnetic field with high spatial variations over a small distance at the interface between the SPIOs and the neighboring intestinal tissue. Consequently, a high-pass filter of the phase image will allow suppression of all slowly varying effects and depict only the effects originating from the susceptibility differences. The high-pass filtering operation consists of two steps: (1) a low-pass filter of the complex image using a Gaussian filter (equal to the image size) with a normalized σ of 0.14 and (2) a complex division of the complex image by the low-pass filtered image on a voxel-by-voxel basis.

Histology

Histological analysis was performed according to standard techniques on the control and treated mice 24 h post injection of SPIO$^+$*E. coli*. Perls' Prussian blue staining was conducted for the detection of iron and a quantitative assessment by estimation of iron-positive cells was performed using a Zeiss Axioplan 2 microscope (Zeiss, Jena, Germany).

Results and discussion

Successful labeling of the *E. coli* with FeraSpin R was confirmed in a gel phantom where a significant shortening of the T_2 relaxation time of the SPIO$^+$*E. coli* was observed (Fig. 1). At 4 h after injection of SPIO$^+$*E. coli* in mice, hypointense signals originating from the SPIOs were observed in the

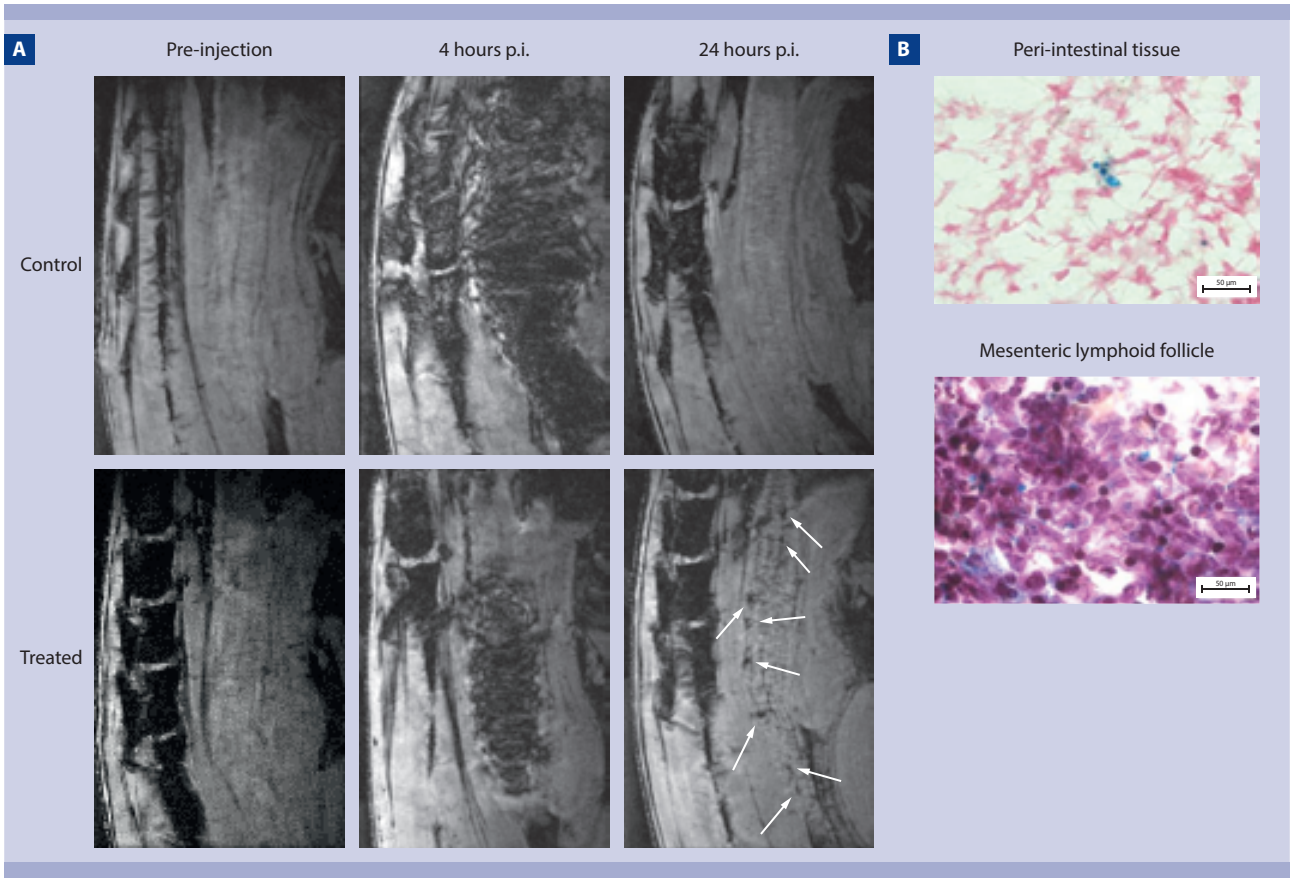

Figure 2: T_2*-weighted MR images and histology images of mice following injection of FeraSpin R-labeled *E. coli* bacteria.
A. Representative MR images of control and treated mice before, 4 h and 24 h after injection of SPIO$^+$*E. coli* into the rectum and lower colon of mice. At 4 h post injection, a hypointense signal originating from the SPIOs was observed in the rectum of all animals. At the 24 h imaging time point this signal was no longer present; however, signals pertaining to the migrated SPIO$^+$*E. coli* were visible in the surrounding tissue of the intestinal wall (arrows). **B.** Histological analysis by Perls' Prussian blue staining of peri-intestinal tissue and mesenteric lymphoid follicles of treated mice 24 h post injection of SPIO$^+$*E. coli* reveals the presence of SPIOs within these tissues.

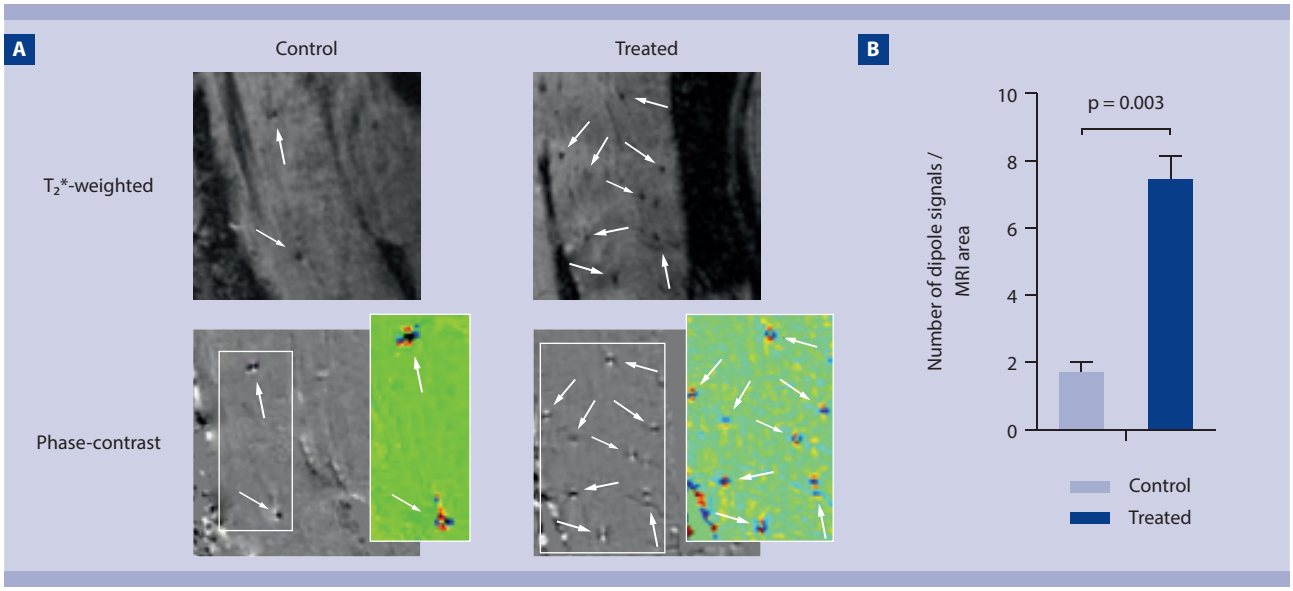

Figure 3: Tracking of FeraSpin R-labeled *E. coli* bacteria in a GvHD mouse model.
A. T_2*-weighted and phase-contrast MR images of the peri-intestinal tissue of control and treated mice 24 h after injection of SPIO⁺*E. coli*. SPIO⁺*E. coli* are more discernible in the high-pass filtered phase-contrast images due to the typical dipole-shaped pattern originating from the SPIOs (arrows, color-coded magnified view). **B.** Quantification of dipole signals in control and treated mice (n = 3 per group) shows significant differences between the animal groups. Data is presented as mean and SEM.

rectum of all animals. At the 24 h imaging time point this signal was no longer present; however, signals pertaining to the migrated SPIO⁺*E. coli* were visible in the surrounding tissue of the intestinal wall (Fig. 2A). These findings were corroborated by histological analysis (Fig. 2B).
Phase-contrast images at the 24 h imaging time point showed that the SPIO⁺*E. coli* gave rise to dipole-shaped signals making them even more discernible than in the T_2*-weighted images (Fig. 3A). Interestingly, these signals were significantly more frequent in the treated mice as compared to the control mice indicating that TBI enhanced transmigration of bacteria through the epithelial layer of the intestinal wall (Fig. 3A and B).

Conclusion

In this study we demonstrate that with phase-contrast MRI it is possible to track the translocation of FeraSpin R-labeled *E. coli* bacteria in mice from the rectum into the peri-intestinal tissue. As expected, this translocation was enhanced in TBI-treated animals as compared to the control. The phase-contrast images depict the small-scale phase variations caused by FeraSpin R which, compared to conventional T_2^*-weighted images, allow for improved monitoring of bacterial migration and their distribution in tissue.

Acknowledgements

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