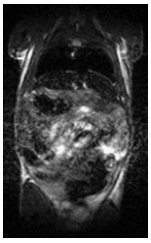
Longitudinal and quantitative cell-distribution studies.

The pEPR cell quantification tests were performed with pre-labeled stem cells. Labeling of the stem cells was performed by ***KULeuven/imec/ReGenesys.*** The magnetic labels were magnetoliposomes (particles under test) developed by KULeuven and imec. And MRI imaging was performed with a 3Tesla MRI of MR-solutions at the Bio-Imaging Lab, ***University of Antwerp***.

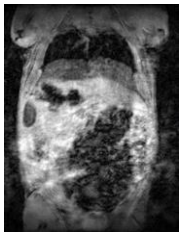
The first pilot study (n=2) was designed as follows:
Particle uptake by the stem cells was documented with histology and ICP-OES. The doubling time of control and labeled cells was checked. And the viability of control and labeled cells was confirmed before administration to the mice. Labeled cells were measured with pEPR as calibration set for ex-vivo quantification.
Control and labeled cells were injected into the tail vain of the mice. In-vivo MRI scans were taken before injection (baseline), 1 day after injection and 7 days after injection. Ex-vivo pEPR quantification on tissue samples was performed on the mice sacrificed at 1 day and 7 days after injections after the in-vivo MRI scans.

T2 weighted image

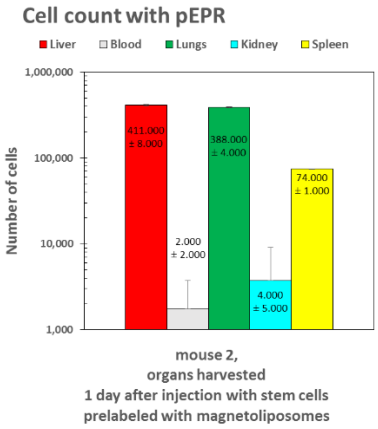


In-vivo MRI of mouse 2,
1 day after injection

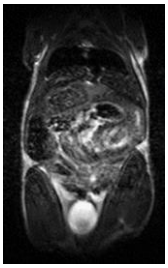
T2 * weighted image



In-vivo MRI of mouse 2,
1 day after injection

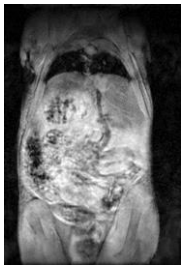


T2 weighted image

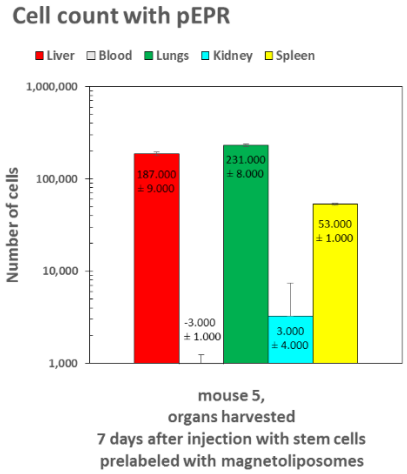


In-vivo MRI of mouse 5,
7 days after injection

T2 * weighted image



In-vivo MRI of mouse 5,
7 days after injection



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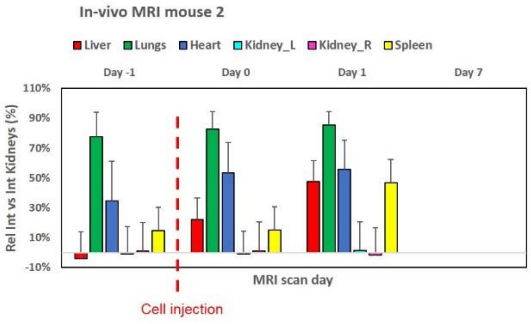
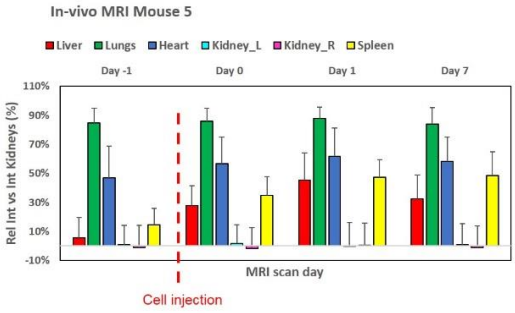
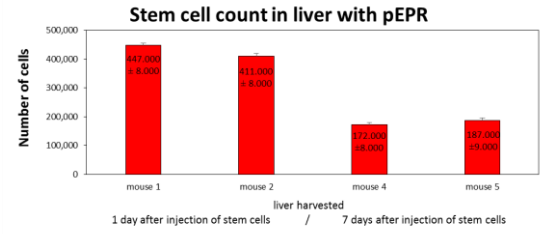
This pilot study as a first try-out resulted already in following preliminary conclusions:

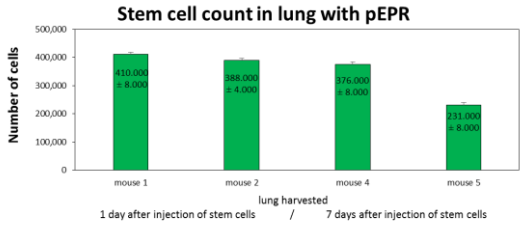
MRI relative intensity changes (T2 and T2*) were measured as function of time. Typically, signal intensity on weighted MRI images is relative and does not allow accurate quantification. No significant cell uptake was detected at the kidneys and therefore, the kidney was selected as reference region for relative quantification. A significant change in intensity was detected in the liver upon injection of labeled cells. For other regions (spleen, heart and lung) changes in MRI signal caused by the migration of labeled cells remained rather inconclusive. This can be ascribed to the limited sample size and the limited accuracy of the MRI method for absolute quantification with the lungs as an extreme example of it. Although the majority of the cells are expected to accumulate in the lungs right after injection, no changes in contrast are detected with MRI due to the lack of protons in the lungs. These initial results illustrate the limitations of MRI for accurate determination of cell kinetics and distribution patterns.

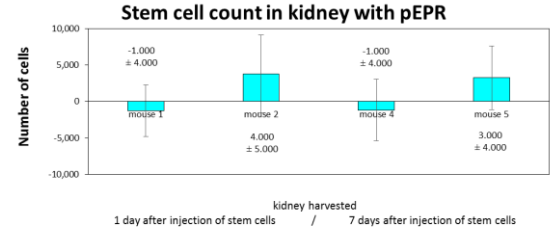


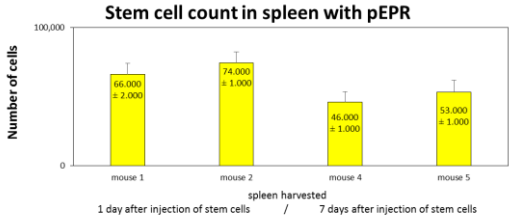


With **pEPR the clearance rate of the cells from the organ tissues** can be determined. The pEPR measurements indicate that on average 58% of the stem cells are cleared from the liver in the timeframe between first and seventh day after cell administration, 24% from the lungs and 30% from the spleen. No cell migration to the kidneys was detectable.









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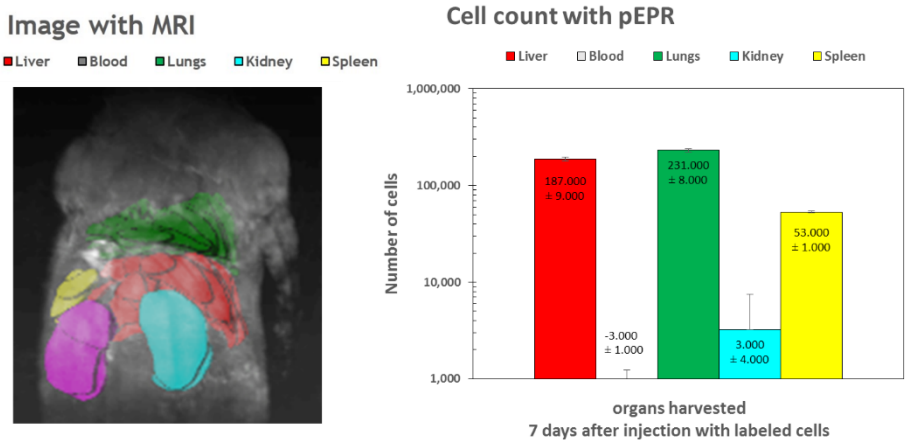
For a **clear visualization of the anatomical images and quantification of the cells**, the MRI data were uploaded in VivoQuant™ enabling 3D imaging and segmentation.



An more systematic and extensive study is ongoing to demonstrate **the cell distribution in tissue, saturation levels and clearance time in-vivo with MRI and ex-vivo with pEPR.**
For this study sufficient time points will be taken to be able to fit the proper clearance curves and this with a better selection of mice (weight, male/female) to eliminate biological variations within the animal population. The migration of the cells to other tissues will be measured. The role of macrophages capturing the cells and/or the particles will be investigated. And a comparison of pEPR with qPCR will be made as a benchmark study.

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