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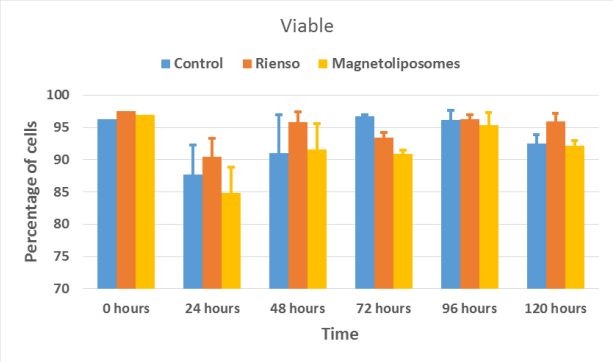
Cell labeling and in-vitro tests - for longitudinal quantitative cell-distribution studies.

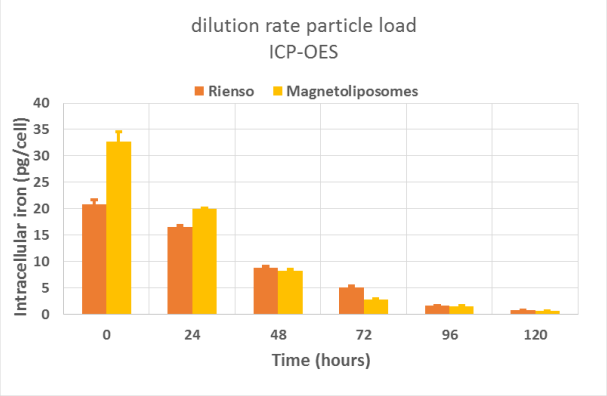
Stem cells were pre-labeled by ***KUL/imec/ReGenesys*** with commercial particles, Rienso®/Feraheme®, and with particles under test, Magnetoliposomes developed by KULeuven and imec.

Over 5 consecutive days the viability of the labeled cells was demonstrated and cell proliferation was monitored for labeled and unlabeled cells.



The proliferation rate of the labeled cells was only a little lower than for un-labeled cells and is considered as acceptable. The average doubling time for control cells is 27 hours, for cells labeled with Rienso 33 hours, and for those labeled with Magnetoliposomes the doubling time was 28 hours.

The dilution rate of the particles or particle load for the different cell generations was determined with ICP-OES.



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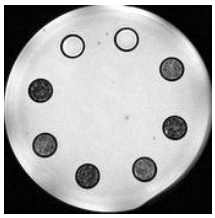
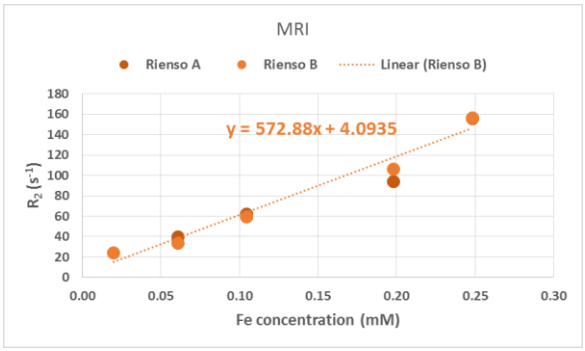
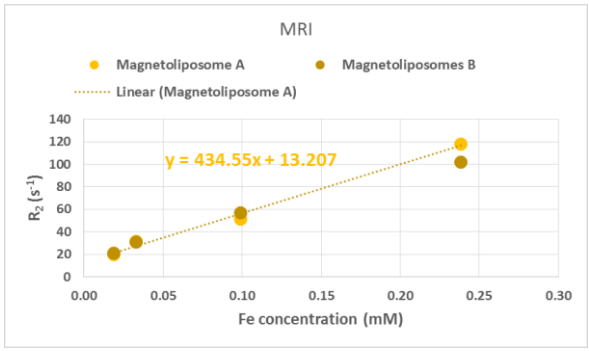
At first, quantification of the labeled cells in phantoms was investigated with T2* relaxivity measurements on a benchtop 3T MRI of MR-solutions, at the Bio-Imaging Lab, ***University of Antwerp***.



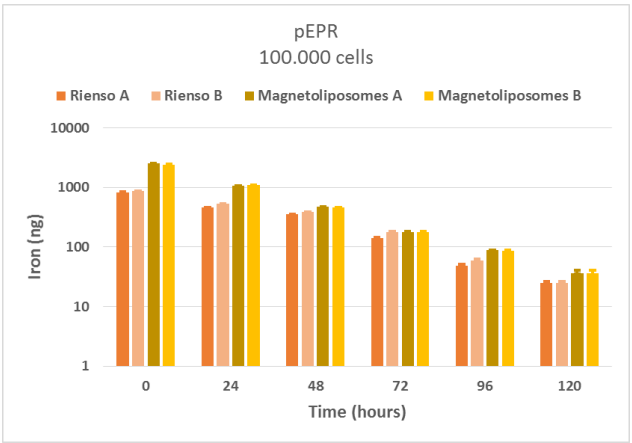
MRI phantom: 100.000 cells in each tube: control cells, cells labeled with Rienso, and labeled with Magnetoliposomes.
Phantoms are made for 5 consecutive days to determine dilution curves, stability measurements and sensitivity limits.

On the **MRI images of the phantoms**, the control cells were not discernable from the labeled cells after 3 days, since particle load was diluted under the sensitivity limit of the MRI system of 0.05mM(Fe) or 400ng(Fe) in 150µL. Also quantification with MRI can be disturbed by irregularities in cell and particle environment as a consequence of the indirectness of the contrast, and upper quantification limits of MRI are determined by saturation or blackening of image.





Then, **with pEPR** the labeled cells in phantoms could be measured over all 5 days, with the detection limit of the pEPR system of about 1.2µM(Fe) or 10ng(Fe) in 150 µL.
This indicates that with a particle load of 20 to 30 pg(Fe)/cell, some 1000 cells in a detection volume of 150µL are detectable with pEPR.



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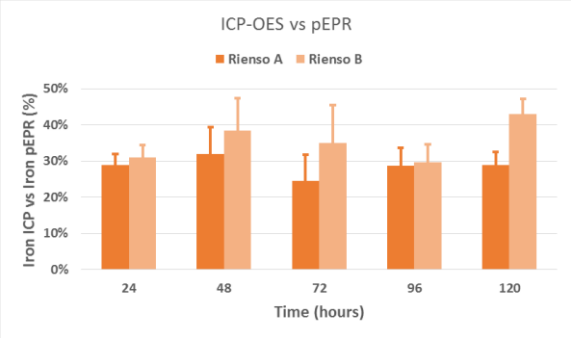
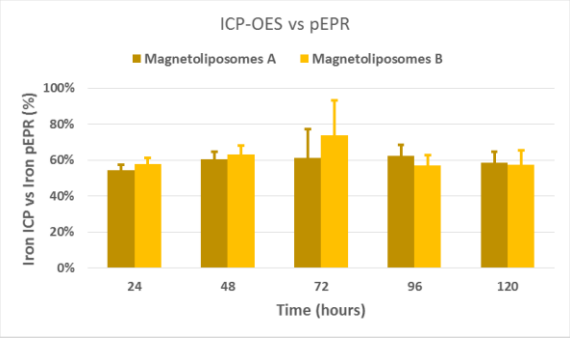
To demonstrate the **longitudinal monitoring of cells with pEPR** it is equally important to determine if there is an interaction between cell and particle influencing the particle signal, e.g. induced by proliferation of the cells or caused by digestion of particles within the cell.

Stability measurements were performed: the T2* signal of a sample with a constant amount of particles, this is with a growing amount of cells, was stable within the accuracy of the signal intensity over the 5 consecutive days, indicating that the proliferation did not affect the labeling except for a dilution over the different cell generations.
Same stability measurements were performed with pEPR and ICP-OES, also indicating that within the accuracy the amounts detected by pEPR were stable compared to the amounts measured with ICP-OES, demonstrating that the magnetization of the particles is not affected over time by cell-particle interactions.





The measurements also demonstrate that with pEPR the signal intensity is proportional to the particle load, however compared to ICP-OES the signal strength decreases with a factor of 2 to 3 from the moment that the particles are incorporated in the cell. Since this factor remains stable over time, longitudinal quantification of labeled cells is possible with pEPR.

An 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.**

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