Longitudinal follow up of pancreatic islets by MRI using labeling with magnetoliposomes

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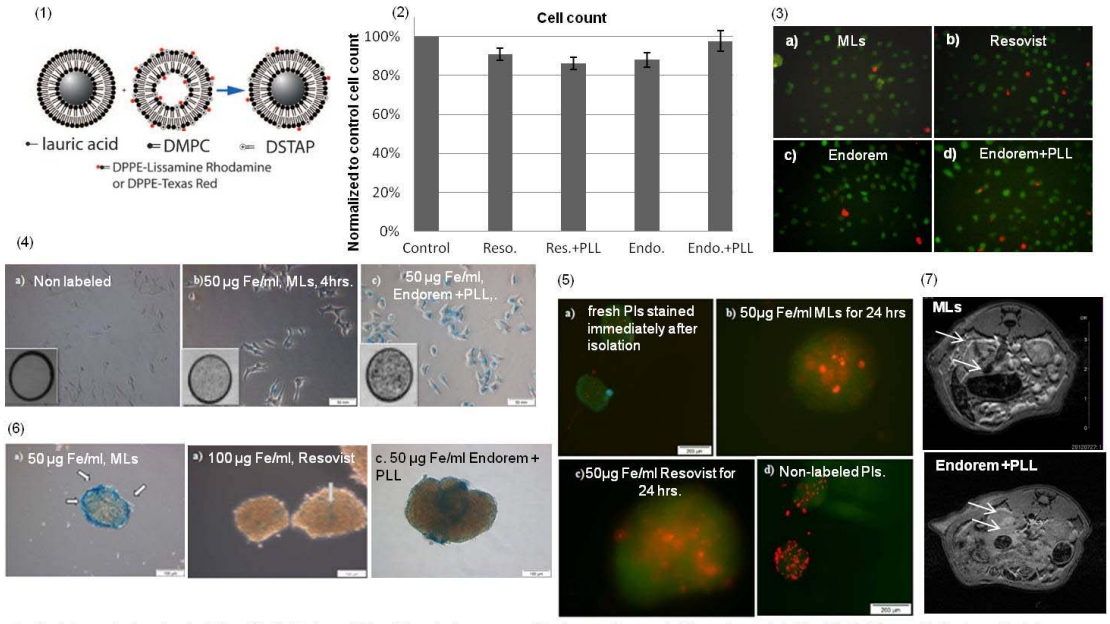
Target audience:
The presented data are of interest for researchers working with animal models of diabetes and pancreatic islet implantation.

Introduction: Transplantation of Pancreatic Islets (PIs) have been considered an alternative therapy for patients with type 1 diabetes mellitus (T1DM). However, graft rejection is a common complication. The exact location of the islet graft after implantation in the liver or abdomen is usually unknown and makes it difficult to assess the fate of islet grafts post transplantation. Thus use of a non invasive, reproducible and sensitive imaging technique is critically needed in order to determine possible causes for the graft rejection. Until now, some studies already indicate the location of PIs labeled with commercially available contrast agents in clinical studies (1). Disadvantages of those labeling methods are the long time it requires for prelabeling PIs and subsequent potential adverse effects on PI functionality as well as the fact that those agents are not available any longer (2). This surges the necessity of determining a replacement CAs for such tracking studies. In this study, we have compared uptake efficiency of cationic magnetoliposomes (MLs) with Endorem and Resovist by labeling insulinoma cell lines and freshly isolated rat PIs.

Methods: Pancreatic Islets**:** Pancreatic islets were isolated from female Sprague Dawley rats (body weight 175 -299 g). Collagenase induced digestion was done and islets were handpicked and counted.
In Vitro studies: Insulinoma (INS-1), Brin-BD11 cells and freshly isolated rat islets were labeled with Resovist, Endorem (+/- PLL) and cationic MLs at 50 µg Fe/ml for 4- 72hrs. MLs were synthesized as described in (3). All labeled samples were collected for high resolution MR scanning, viability testing (FDA-Propidium iodide staining), electron microscopy and prussian blue staining (for label confirmation) and ICP-MS (for iron quantification). Functional assays like insulin secretion and insulin content were performed post labeling.
In vivo experiments**:** In order to assess if the *in vitro* optimized labeling conditions result in sufficient *in vivo* MRI detectability, healthy rats received freshly isolated islets, labeled with all particles. Labeled islets were transplanted in the kidney capsule. Islets were monitored with MRI longitudinally (up to 1 week) . Further kidneys were collected for histological confirmation with H &E and prussian blue staining.
MRI: MR images were acquired from agar phantoms containing labeled cells/ PIs and from rats using a 9.4T Biospec small animal MRI scanner (Bruker Biospec, Ettlingen, Germany). T2-weighted spin echo and gradient echo images were acquired (TE= 12 ms, TR= 150 ms.).Respiration gating was applied for *in vivo* experiments

Results:INS-1 labeled with different labeling conditions showed varying uptake with different particles. MR detectable and non toxic uptake was achieved when MLs were incubated only for 4hrs. whereas Resovist and Endorem (+/- PLL) needed either longer duration or addition of PLL to achieve the same. No particles showed detectable toxic effects post labeling on insulinoma cells. In contrast, efficient labeling for freshly isolated PIs, was only achieved after 72 hrs of co-incubation with Resovist. Only cationic MLs showed very good uptake after 24hrs with low labeling concentrations of 50 µg Fe/ml without affecting insulin secretion and the viability. Longitudinal detection of MLs labeled cells and PIs was possible in *in vivo* in rats with 3D T2* w MRI.



Typical layout of cationic MLs (1); INS-1 and PIs did not show any effect on cell count (2) and on viability (3) & (5) post labeling; Uptake was confirmed with prussian blue staining (4) & (6); islets labeled with MLs were detected in kidney capsule in the form of hypoitense spot (white arrow) which was not the case wit hEndorem + PLL labeled PIs.

Conclusion:
MLs were incorporated in shorter duration compared to commercially available CAs. Also it was observed that MLs are safe CAs for longitudinal and reproducible *in vivo* visualization of engrafted pancreatic islets in rodent models.

References:

(1) Jirak D.*et.al.* MRIM, 2004; (2) Cromer-Berman S. WIREs Nanomedicine and Nanobiotechnology, 2011; (3) Soenen S., *et.al.*ChemBioChem 2009