

# Magnetoliposomes open up new horizons as MRI contrast agents.

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## Introduction

High resolution MRI has a low sensitivity for the visualization of low cell numbers in vivo. This is circumvented by the internalization of high concentrations of superparamagnetic iron oxide particles resulting in potential cytotoxic effects or affecting cell biology like differentiation, formation of ROS etc. [1]. In addition, the ability to monitor iron oxide labelled cells in vivo is often hampered by a limited stability of the particle coating [2]. In the present work, we have investigated magnetoliposomes (ML) for stable, non-toxic, longitudinal cell labelling and monitoring.

## Material and Methods

**Synthesis:** Neutral and anionic MLs were synthesised as described [3]. Cationic magnetoliposomes (MLs), i.e. 14-nm diameter iron oxide cores each individually wrapped by a lipid bilayer containing 3.33% of distearyltrimethyl ammoniumpropane (DSTAP) were synthesised in a two-step procedure as described in [4].

**Cell culture and toxicity studies:** MLs were compared with Resovist, Endorem (dextran-coated) and VSOP (citrate-coated) iron oxide particles. Neural progenitor cells (C17.2), rat pheochromocytoma cells (PC12) and human blood outgrowth endothelial cells were labelled with the MLs and control particles. Apart from uptake and stability, the following parameters were investigated to assess potential cytotoxicity: survival, proliferation, cell morphology and cytoskeleton (staining for F-actin, alpha-tubulin, and vinculin), detection of reactive oxygen species (ROS) formation and functionality (neurite formation, transferrin-receptor 1 expression etc.).

As nanoparticles are usually stored in endosomes and lysosomes, the pH-dependent stability of the particles was correlated with intracellular particles stability (TEM and [Fe]). In addition, the effect of exposure of MLs to phospholipases on the ML stability was studied. Intracellular particle clustering was evaluated by MRI (T1/ T2/ T2\*).

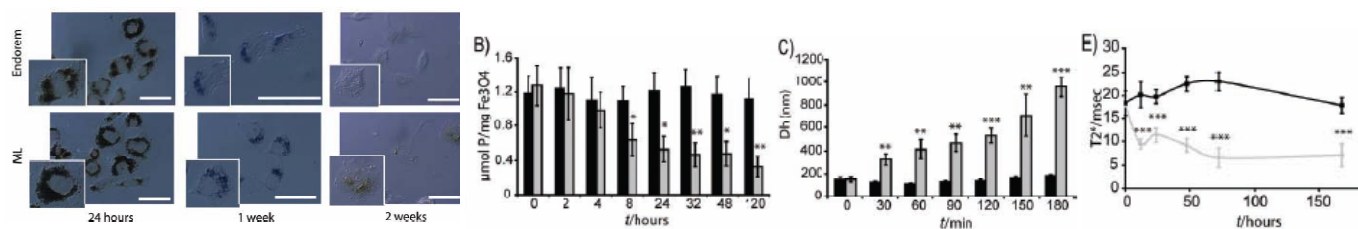
**MRI:** Cells were suspended in agar phantoms for MRI. All MR images were acquired using a Bruker Biospec 9.4 Tesla small animal MR scanner (Bruker Biospin, Ettlingen, Germany; horizontal bore, 20 cm) equipped with actively shielded gradients (600 mT m<sup>-1</sup>). A quadrature RF resonator (transmit/receive; inner diameter 7 cm) was used to acquire 2D multi-slice-multi-echo (MSME) experiments for the calculation of T2-maps (TR=3,000 ms and 16 TE increments of 10 ms, 275 µm in plane resolution, 0.35 mm slice thickness) and 2D multi-gradient echos for the calculation of T2\* maps (16 TE increments, first TE=4.5 ms with increments of 6.7 ms).

## Results and Discussion

Iron uptake and intracellular stability was highest with positively charged MLs. Citrate-coated particles were degraded rapidly, whereas dextran-coated ones are more stable, but still less than the lipid-coated MLs. The degradation of the particles was shown by the increase in free ferric ions, and the distorted r1/r2 ratio of the particles, hampering their use for long-term cell imaging. Cells labelled with the different particles reaching similar amounts of intracellular iron, show increases in ROS formation and transferrin receptor expression in C17.2 neural progenitor cells and impeded functionality of PC12 rat pheochromocytoma cells. The extent of these effects is in line with the degradability of the particles in vitro. The MLs appear to be the most stable particles and further show a high persistence of the label in continuously proliferating C17.2 cells. When exposed to phospholipases, which degrade lipid structures, the outer lipid layer is degraded, but the inner lipid layer is strongly resistant due to an unfavourable orientation of the lipids. This leads to monolayer coated iron oxide cores which tend to aggregate in cultured cells. The clustered particles have a much stronger cumulative magnetic dipole and result in a significant shortening of transverse relaxation. This makes MLs ideal MR contrast agents as their small size enables efficient cellular uptake, and the subsequent intracellular clustering enhances their MR contrast generation.

## Conclusions

The results indicate the type of coating material used is highly important with regards to maintaining cell functionality and stability of the label. MLs have the advantage of low toxicity and high stability. Intracellular clustering decreases the iron concentration needed for cell detection.



**Figure 1:** Clustering of ML after exposure to PLA2: (A) C17.2 cells labelled with Endorem and MLs indicate longer detectability after labelling with MLs due to intracellular clustering. (B) ratio of phosphate to magnetite decreases in ML after exposure to PLA2 (gray) but remains stable at 1/3 indicating a stable inner phospholipid layer. (C) This results in intracellular clustering as indicated by the hydrodynamic diameters of PLA2 treated MLs (gray) compared to untreated MLs (black). This is also reflected by decreased T2\* values of PLA2 treated MLs (D).

**References:** [1] Soenen SJ & De Cuyper M. *Contrast Media Mol. Imaging* 4: 207 (2009). [2] Wilhelm C & Gazeau FJ. *Biomaterials* 29: 3161 (2008). [3] Soenen SJ et al. *Nanomedicine* 4: 177 (2009). [4] De Cuyper M et al. *Methods Mol. Biol.* 605: 97 (2010).