The effect of intracellular clustering on the stability and contrast generating properties of SPIOs: a comparison between PEGylated SPIOs and liposome SPIOs

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Introduction

Superparamagnetic iron oxide nanoparticles (SPIOs) are often used as MRI contrast agent for cell labeling to enhance the sensitivity of high resolution MRI. After internalization, the SPIOs are transferred to endosomes and lysosomes [(1)]. Intracellular clustering of the SPIOs can occur because of the interaction of the coating of the SPIOs with the endosomal environment [(2)]. In the present work, we have compared the uptake, toxicity, stability and contrast generating properties of non-clustering PEGylated SPIOs and clustering lipid bilayer SPIOs (magnetoliposomes).

Material and Methods

Synthesis: Monodisperse SPIOs were synthesized through a thermal decomposition method and a seed mediated growth method as described [(3)]. In a next step these monodisperse cores were coated with 1) a PEGylated-silane molecule to produce PEGylated-SPIOs and 2) enwrapped by a lipid bilayer to create liposome-SPIOs. For fluorescent confocal microscopy a FITC-PEGylated silane was used and a rhodamine labeled phospholipid was incorporated in the lipid bilayer.

Cell culture: Mesenchymale stem cells (MSCs) were exposed for 24 hours to different concentrations of the two SPIO types. After an overnight SPIO free incubation, the cells were harvested for further study. The initial toxicity of the SPIOs on the cells was investigated by cell count and by a cell viability assay (Alamar blue and Prestoblue). SPIO uptake was quantified by digesting the labeled cells and measuring the iron content using ICP-OES. Uptake was further evaluated by Prussian blue staining, fluorescent confocal microscopy and transmission electron microscopy (TEM) after cell fixation. A certain amount of cells was replated and kept in culture for several days to evaluate long time effects.

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-1). 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 multigradient echos for the calculation of the T2* maps (8 TE increments, first TE=4.5 ms with increments of 6.7 ms).

Results and Discussion:

Monodisperse SPIOs were successfully synthesized using the thermal decomposition and seed mediated growth method (~12.5nm σ<10%). In a second step the initial hydrophobic coating was exchanged for a PEGylated-silane or liposome coating, creating biocompatible SPIOs with a similar hydrodynamic size (~40nm). Uptake in cells was larger for liposome coated SPIOs (max. ~40pg Fe/cell) versus the PEGylated-SPIOs (max ~15 pg Fe/cell) which agrees with the properties of a non-interacting PEGylated coating. Limited toxicity was seen in the higher exposure concentrations (200 µg Fe/ml), however no toxicity was observed in the cell viability assays. The SPIO uptake was further confirmed by fluorescence confocal microscopy, which clearly indicated a perinuclear localization of the SPIOs after 24 hour incubation. In addition TEM showed the preservation of the monodispersity of the SPIOs after cell uptake and the accumulation of the SPIOs in vesicles, most likely to be endosomes or lysosomes. Most importantly an obvious clustering of the liposome-SPIOs can be seen in the TEM images, which cannot be seen in the TEM images of the PEGylated-SPIO labeled cells. A fixed amount of cells (500 cells/µl) was suspended in an agar phantom and the contrast generating properties were evaluated. Maximum signal loss was obtained at about 20 pg Fe/cell and both SPIOs have similar lowest amount of detection. No initial difference in contrast could be seen when comparing the cells labeled with PEGylated-SPIOs and the cells labeled with liposome-SPIOs. However when looking at the contrast generating properties over longer time, the liposome-SPIOs perform better, what can be attributed to the increased stability of the clustered liposome-SPIOs.

Conclusion:

From these results we can conclude that the coating material greatly determines the intracellular faith and stability of the SPIOs. We could not conclude that the clustering of SPIOs results in a significant shortening of transverse relaxation at comparable iron content. However the increased stability of the liposome-SPIOs makes them highly suitable as cell label for long-time monitoring.

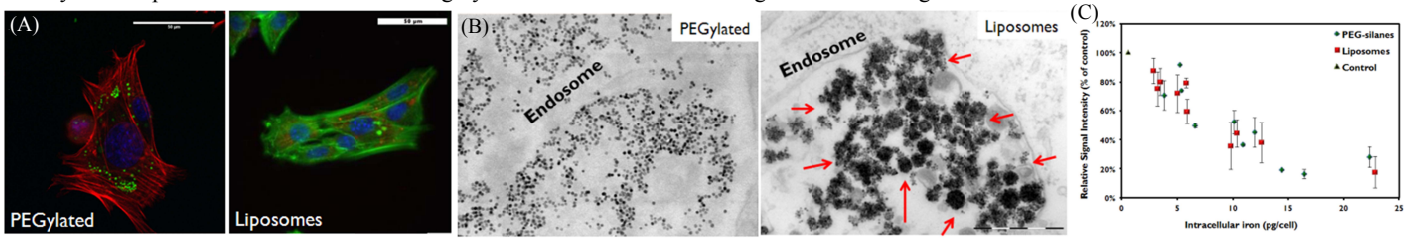

Figure: (A) Confocal microscopy images of MSCs labeled with PEGylated-SPIOs (left) and liposome-SPIOs (right). (B) TEM images of an endosome filled with non-clustered PEGylated-SPIOs (left) and clustered (red arrows) liposome-SPIOs (right). (C) Graph of relative signal intensity (% of control) based on MR images versus the intracellular iron uptake.

References: [1] Becker C. et al., JMMM 311, 234 (2007) [2] Soenen S.J. et al., Biomaterials 32, 195 (2011) [3] Sun S. et al., J. Am. Chem. Soc. 126, 273 (2004)