Magnetoliposomes open up new horizons as MRI contrast agents

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Over the past few decades, the interest in magnetizable nanocolloids has steadily increased as a result of the growing number of applications which can be designed both in the field of biotechnology and biomedicine. To improve the colloidal stability the particles have to be wrapped in a cushion of adsorbing molecules such as dextrans which are often used for the production of magnetic resonance imaging (MRI) agents. However, the dextran coating is vulnerable to aging and may induce alterations in the behavior and morphology of cells in culture. As a promising alternative we introduced in the late 1980's so-called magnetoliposomes (MLs) [1]. These constructs consist of nanometer-sized magnetite (Fe_3O_4) cores covered with a bilayer of phospholipid molecules. The inner leaflet molecules are very strongly chemisorbed with their polar headgroup on the Fe_3O_4 surface, whereas those residing in the outer layer are more loosely physisorbed. Since phospholipids are components of all biological membranes, MLs can be considered as highly biocompatible, which is essential, for instance, for *in vivo* cell labeling [2].

In the present work we further exploit the high flexibility of the lipid bilayer, enabling easy and well controllable **particle functionalization**. In this respect it is shown that ***cationic lipids*** built in the outer leaflet of the ML envelope, ultimately, result in much higher cellular uptake [3,4]. For instance, using 3.33 mol% 1,2-distearoyl-3-methylammonium propane [DSTAP] containing MLs, 3T3 fibroblasts can be efficiently labeled with up to 47.66 pg Fe/cell, one of the highest values reported in literature, without inducing any toxic effects [5]. Also, upon incorporating 1 mol% of galactose-modified phospholipids into the ML coat, the particles are ***specifically targeted*** and taken up by HepG2 (hepatocellular carcinoma) cells and primary hepathocytes, which typically express the complimentary asialoglycoprotein receptor [6]. In vivo experiments have also shown better retention in the liver of functionalized MLs compared to non-functionalized ones.

Besides our attempts to improve (specific) particle uptake, the **intracellular fate** of the MLs is further investigated and compared with the behavior of other well known MRI contrast agents. As, in general, internalized particulates end up in the 'acidic' endosomes/lysosomes, we first studied nanoparticle susceptibility to **pH-induced degradation** and the consequences thereof for MR contrast generation. Kinetic experiments were performed in a test tube setup at pH 7.0, 5.5 and 4.5. Both by Fe^{3+} determinations and analysis of T2* maps of MRI phantoms it is found that **MLs withstand much better the harsh acidic conditions** as compared to Endorem (dextran-coated), Resovist (carboxydextran-coated) and VSOP (citrate-coated) particles [7].

Besides the above-mentioned pH-induced effect, the lipid bilayer is also partially destroyed by the action of lysosomal phospholipase A2. The kinetics of this enzymatic degradation are monitored in an *in vitro* lysosomal model system. A gradual decrease in pH caused by liberated fatty acids (Fig. A) as well as a concomitant lowering of the phospholipid/iron ratio of the MLs is observed (Fig. B). At equilibrium the remaining lipid amount on the Fe_3O_4 core equals about one third of the value found for an intact ML. This observation strongly points to the fact that the inner lipid layer is very resistant to further hydrolysis due to an unfavorable orientation and, thus, avidly protects the iron oxide core. Monolayer-coated particles, indeed, display a hydrophobic surface and – due to the hydrophobic effect

- will **cluster** in the aqueous environment, resulting in a 6-fold increase in hydrodynamic diameter (as measured by dynamic light scattering – Fig. C), an enhanced magnetic attraction in the presence of an external magnetic field (Fig. D), and a 2-fold reduction of T2* relaxation time [7]. This aggregation event observed with MLs also occurs intracellulary as shown with C17.2 neural progenitor cells. By contrast, with Endorem, at a similar initial intracellular iron oxide level, no aggregation occurs [7].

In conclusion, MLs show up as highly versatile, biocompatible nanocolloids which are extremely prone for **long-term MRI follow-up studies**.

Acknowledgements

This research is supported by SBO project nr IWT/80017 to MDC and UH. SJHS is a postdoctoral fellow from the FWO-Vlaanderen. UH received financial support from EC-FP7 ENCITE and the KUL program financing IMIR

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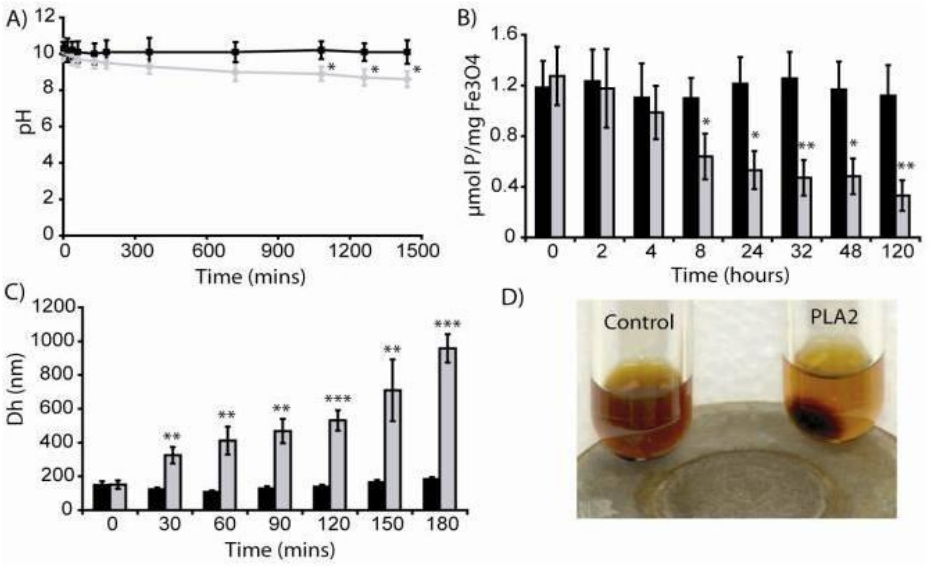
Figures



Effects of PLase A_2-action on MLs, shown by A) the decrease in pH, B) the ratio of phosphate over magnetite, C) increase in hydrodynamic diameter and D) visual confirmation of an incresae in aggregation of PLA_2-treated MLs. A-C) grey indicates PLA_2-treated samples; black represents control samples.