# A macrophage specific nanoparticle suitable for magnetic resonance, fluorescence, and magnetic particle imaging

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## Introduction:

Magnetic particle imaging (MPI), introduced in 2005, is a new imaging modality that allows the direct visualization of magnetic particle concentration with high sensitivity and unprecedented high temporal resolution (1). The advantages of MPI could be exploited for a variety of purposes, particularly in real-time cardiac imaging. The successful application of MPI relies heavily on the properties of the superparamagnetic nanoparticles used. It is of eminent importance that the nanocrystalline characteristics of these particles, i.e. crystalline structure, size and magnetic properties, are judiciously chosen and well defined. Recently, we introduced a nanoparticle platform of which the coating was based on high density lipoprotein (HDL) and the core was comprised of an iron oxide nanocrystal (FeO-HDL) (2). In vitro, this platform was demonstrated to exhibit macrophage specificity and in vivo it was successfully employed for macrophage imaging in atherosclerotic plaques of apoE-KO mice using magnetic resonance imaging (MRI), fluorescence imaging and confocal microscopy.

In the current study we further modified this platform to make it also suitable for MPI by using 20 nm, highly monodisperse magnetic particles as the core of FeO-HDL. We demonstrate preferential uptake by cultured macrophages with confocal laser scanning microscopy (CLSM), MRI, and magnetic particle spectroscopy (MPS).

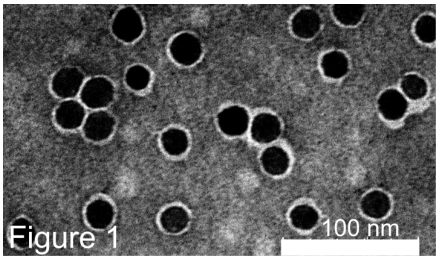

Figure 1

## Methods:

The synthesis of FeO-HDL was achieved by co-dissolving the phospholipids, fluorophore, and ~20 nm oleic acid coated iron oxide nanoparticles in a chloroform-methanol solvent mixture. This solution was slowly added to hot buffer, forming the encapsulated particles. ApoA-I, a protein of HDL that targets macrophages, was included in the phospholipid layer via incubation. Purification was achieved via centrifugation, filtration and washing. PEG-lipid coated particles were also formed and served as controls. These nanoparticles were characterized via (negative staining) TEM, ICP-MS, relaxometry, phosphorous and protein quantification.

J774A.1 macrophage cells were incubated with FeO-HDL, FeO-PEG or left untreated for 1 hour. Confocal laser scanning microscopy (CLSM) was performed on a small subset of DAPI stained cells. The remaining cells were collected as a loose pellet and analyzed using T2-weighted imaging on a 9.4 T MRI scanner and magnetic particle spectroscopy. In MPS the nonlinear magnetic response of the pellets was measured in an oscillating magnetic field (10 mT amplitude, 25 kHz frequency, 30 sec sampling time). The 3rd harmonic MPS signal intensity was monitored to quantify contrast agent uptake in macrophage cells.

## Results and Discussion:

The synthesis and purification of the agent resulted in individually dispersed particles that were encapsulated in a single layer of phospholipids (Figure 1). Protein analysis showed that the apoA-I readily adsorbed onto the particle. In Figure 2 CLSM images show the nuclei of DAPI stained macrophages in blue, the FeO-HDL particles (Figure 2A) and the FeO-PEG particles (Figure 2B) in red. A clear preferential uptake of FeO-HDL as compared to FeO-PEG was observed. In line with these CLSM findings T2-weighted MRI images of the different cell pellets also indicated elevated uptake of the FeO-HDL as compared to the controls (Figure 2C). Since MPS allows the quantification of magnetic particles we confirmed the aforementioned findings with this technique. In Figure 2D the 3rd harmonic intensity of magnetic particle spectra is plotted for cell pellets that were incubated with FeO-HDL, FeO-PEG and a control cell pellet. A significantly higher uptake of FeO-HDL was observed.

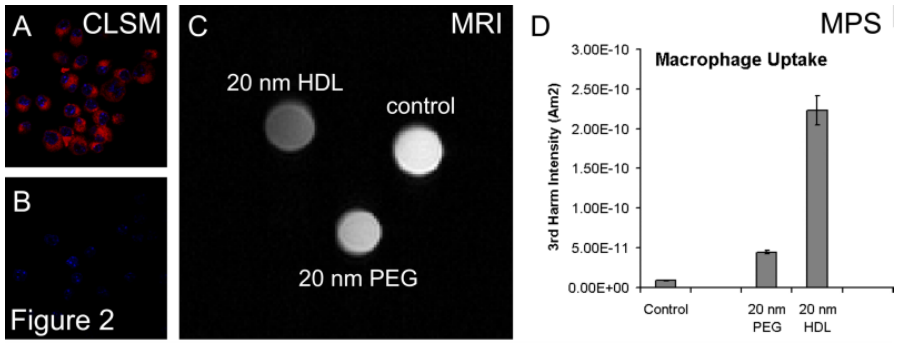

Figure 2

## Conclusions

In conclusion, we have synthesized a novel type of nanoparticulate contrast agent for macrophage-specific multimodality imaging. Macrophage cells avidly took up the nanoparticle in vitro as evidenced by confocal microscopy, magnetic resonance imaging and magnetic particle spectroscopy. This study, for the first time, demonstrates the feasibility of magnetic particle imaging of macrophage cells and potentially opens the opportunity for diagnosis of cardiovascular disease in general and macrophage plaque imaging in particular.

(1) Gleich and Weizenecker.Nature. 2005 Jun 30;435(7046):1214-7. (2) Cormode et al. Nano Lett. 2008 DOI: 10.1021/nl801958b