# Comparison of SPIO and USPIO for in vitro labelling of human monocytes with respect to MR detection and cellular activation status

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## Introduction

Lesion formation in multiple sclerosis (MS) is characterized by infiltration of immune cells into the brain, ultimately leading to destruction of myelin sheets. Monocyte-derived macrophages play a crucial role in the pathogenesis of MS [1]. Cell imaging by magnetic resonance (MR) may be a powerful tool to non-invasively monitor therapies that affect cell migration into the central nervous system (CNS) during MS. Prior studies have evaluated the use of ultra small superparamagnetic particles of iron oxide (USPIO) as MR contrast agents to reveal lesion formation and to label monocytes in vivo [2,3]. However, it remains unclear whether USPIOs are taken up specifically by monocytes and whether uptake occurs in the circulation or after accumulation in the CNS. Incubating freshly isolated monocytes with iron oxides and re inject them into circulation may be a better tool. To this end we labelled primary human monocytes (PHM) in vitro, comparing two frequently used (U)SPIOs and assessing the effect of iron incorporation on cell activation status. Tracking cells of interest in vivo using MR imaging requires selective cell labelling without affecting cell viability and activity. Ultimately, this procedure will be used to track in vitro labelled rat monocytes in an animal model for MS.

## Material and Methods

PHMs were obtained by Lymphoprep density centrifugation of four healthy donors and purified using immunomagnetic beads coated with a monoclonal antibody directed against the monocytic CD14 molecule (MACS-CD14; Milteny, Biotec). Subsequently, monocytes were incubated for 1.5h at 37 $^{o}$C with SPIO Endorem (~150 nm) or USPIO Sinerem (~30 nm) at concentrations ranging from 0.1 - 3.7 mg Fe/ml/$10^6$cells in culture medium (supplemented RPMI-1640). To evaluate the effect of transfection agents (TAs) in cell labelling, both iron oxides were pre-incubated with different TAs; Fugene (0.1% v/v, 60 min), Poly-L-lysine (1 µg/ml, 60 min) and Superfect (0.05% v/v, 10 min). Uptake of iron was determined by $T_2$-relaxation time MRI performed on a 4.7 T horizontal bore spectrometer (Varian Instruments, Palo Alto, USA). $T_2$-maps were recorded with a multi echo multi slice sequence (9x 0.5 mm, TR=3.2 s, echo-spacing=17.5 ms, echo train length=10, NEX=4). Images were acquired from agarose-gel (0.4 %) suspended monocytes (0.5 x$10^6$ cells). The presence of intracellular iron was microscopically analyzed by Prussian blue staining. Monocyte viability and migratory capacity after labelling was monitored [4]. Levels of the pro-inflammatory cytokines interleukin (IL) -1 and -6 were measured 24h after labelling using standard ELISA. Data were evaluated by two-way ANOVA, followed by a multiple comparison procedure (SNK-Method). P <0.05 was considered statistically significant.

## Results

Isolation with MACS beads did not change the $R_2$ of PHMs (data not shown). PHMs incubated with increasing concentrations of SPIO Endorem clearly showed increasing signal loss ($T_2$-maps, Fig. 1A) and thus a significant increase in $R_2$ (Fig. 1B). In contrast, monocytes incubated with USPIO Sinerem did not show a significant increase in $R_2$ as compared to unlabeled monocytes. Pre-incubation of (U)SPIOs at 1 mg Fe/ml with TAs did not increase the $R_2$ value (data not shown). No difference was found in cell viability and migratory capacity of monocytes incubated at Endorem 1 mg/ml as compared to non-labeled cells (data not shown). Intracellular iron had no effect on the production of IL-1 and IL-6 24h after labelling (Fig. 2).

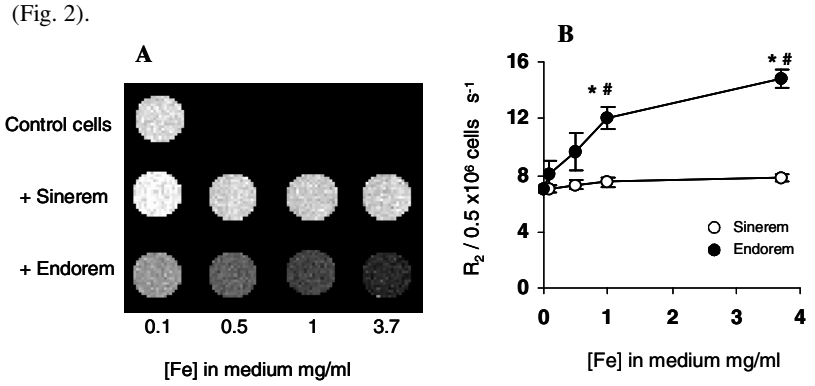


**Fig. 1**: (**A**) Typical example of $T_2$-maps of cells incubated with (U)SPIOs. (**B**) $R_2$ values, * p <0.05 versus Sinerem, # p <0.05 versus unlabeled controls.

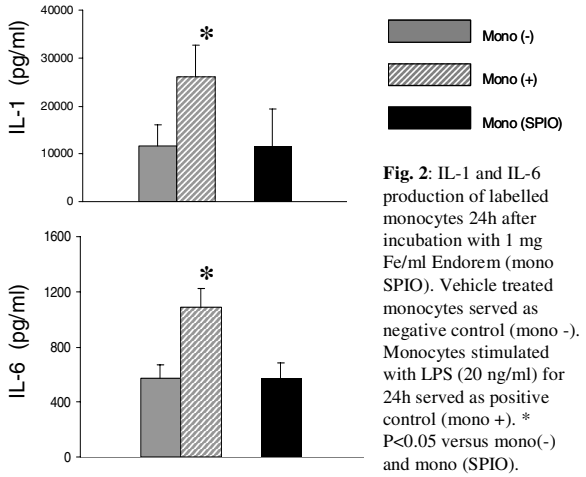


**Fig. 2**: IL-1 and IL-6 production of labelled monocytes 24h after incubation with 1 mg Fe/ml Endorem (mono SPIO). Vehicle treated monocytes served as negative control (mono -). Monocytes stimulated with LPS (20 ng/ml) for 24h served as positive control (mono +). * P<0.05 versus mono(-) and mono (SPIO).

## Discussion

Our data suggest that Endorem is the most suitable iron oxide to label PHMs in vitro. An incubation time of 1.5 h and Endorem at a concentration of 1 mg Fe/ml appeared to be optimal with respect to maximal SPIO uptake and the presence of intracellular iron appeared to have no effect on cellular activation status. In contrast, incubation with Sinerem at a high dose of 3.7 mg Fe/ml results in no significant MR signal change. Pre-incubation with TAs Poly-L-lysine, FuGene or Superfect did not increase the labelling efficiency of both iron oxides. It is probably the larger size of the Endorem particle that favours the observed endocytosis by PHMs. Based upon the results of this study we suggest that labelling primary monocytes in vitro with Endorem shows great potential for in vivo tracking of monocytes with MRI. We are currently implementing the obtained results to label primary rat monocytes and visualize their migratory patterns in vivo.

## References

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