New biodegradable multimeric MPIO contrast agent shows rapid in vitro and in vivo degradation and high sensitivity contrast

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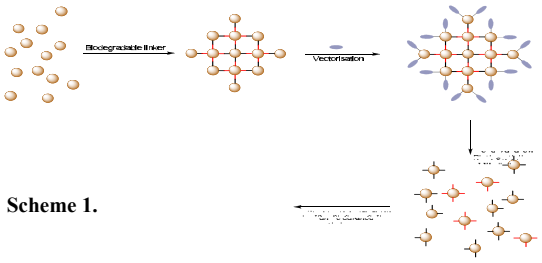
Introduction: Targeted microparticles of iron oxide (MPIO) have been used with MRI to detect pathologies such as multiple sclerosis, stroke, atherosclerosis and cerebral malaria in animal models[1-5]. Despite their successful use in animal models the polystyrene coated MPIO currently used (Dynalbeads) suffer from a lack of biodegradability and poor clearance, which excludes their medical use. Previously, we reported the synthesis of 1μm diameter biodegradable multimeric microparticles of iron oxide (mMPIO), composed of multiple iron oxide nanoparticles (NPs) coupled through a protease labile peptide linker (Scheme 1)[6]. We also showed their optimal MRI contrast properties, stability and *in vitro* degradation by proteases. Herein, we now demonstrate their degradation by cultured macrophages, their biodistribution following intravenous injection, their faster clearance rate from the body in comparison to equivalently sized monomeric MPIO and their use *in vivo* for detection of brain inflammation.



Scheme 1.

Materials and Methods: Multimeric MPIO synthesis: as described previously[6], reaction of of HOOC-NPs (preactivated with EDC and sulfoNHS) with H_2N-peptide-NPs generated mMPIO of *ca*. 700nM. Monomeric MPIO synthesis: co-precipitation of iron oxide in the presence of dextran, followed by epiclorohydrin treatment and ammonia addition furnished *ca*. 700nM amino-terminated monomeric MPIO. *In vitro* macrophage degradation: mMPIO and monomeric MPIO fluorescently labeled with AlexaFluor 488 were added to human macrophages and images obtained using a cooled monochrome QICAM coupled to an Olympus IX-71 inverted microscope. Antibody conjugation to mMPIO (VCAM-mMPIO): Purified monoclonal rat antibodies to mouse VCAM-1 were conjugated to mMPIO by the EDC/sulfoNHS methodology. *In vivo* MRI: Male NMRI mice were injected intracerebrally with 50ng of IL-1β in the left striatum. After 4h animals were injected i.v. with VCAM-mMPIO (4mg iron/kg). 1.5h after VCAM-mMPIO injection animals underwent MRI at 7T. A T2*-weighted 3D GE dataset was acquired (isotropic resolution *ca*. 90μm).

Results and Discussion: 700 nm mMPIO were chosen as they are cleared rapidly (within 30min) from the circulation, thus ensuring efficient particle binding to the site of interest but low background signal at the time of MRI scan. In solution the mMPIO showed no tendency to sediment over time, whereas equivalently sized monomeric MPIO rapidly sedimented out of solution.

Incubation of fluorescently-labelled mMPIO and monomeric MPIO with human macrophages demonstrated that the mMPIO were rapidly taken up by macrophages and subsequently degraded within 24-48h, whilst the large monomeric particles were more slowly taken up and showed little degradation even after 72 h.

In vivo experiments showed that by 1h after intravenous injection the untargeted mMPIO had mainly acumulated in the liver and spleen, with no evidence of retention in brain, lung or heart. Interestingly, the monomeric MPIO showed substantial accumulation in lung. By 7 days after injection, the mMPIO had been entirely degraded in the liver, whilst the 700 nm monomeric MPIO were cleared much more slowly from the liver and not at all from lung (Fig. 1).

Following intraceberal IL-1β injection in the mouse *in vivo*, numerous focal hypointensities were visible in the left striatum that were not evident in either the contralateral hemisphere or a saline injected animal (Fig. 2). These findings indicate specific binding of VCAM-mMPIO to acutely activated endothelium.

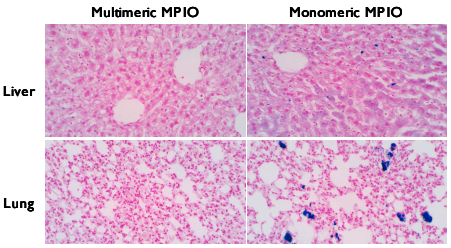


Figure 1: Photomicrographs of sections from organs at 1h and 7 days after i.v. injection of either multimeric or monomeric MPIO. Tissue sections stained with Prussian Blue for iron; counter-stained with neutral red. Mag. x40.

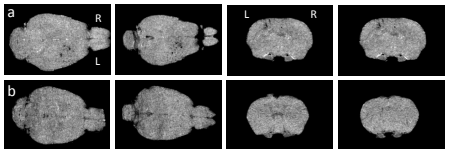


Figure 2: *In vivo* T2*-W horizontal and coronal images from 3D data sets with *ca*. 90μm isotropic resolution. Mouse injected intracranially (left hemisphere) with (a) IL-1β and (b) saline. L = left; R = right.

Conclusion: These new multimeric MPIO are degraded by macrophages in culture and cleared from the body *in vivo* by the pathway described for clinically approved iron oxide nanoparticles. VCAM-mMPIO specifically bind to activated endovascular endotelium providing MRI contrast. Our findings suggest that these mMPIO constitute an ideal platform for new MRI contrast agents.

References: **1.** McAteer *et al* (2007) *Nat. Med.*. 13:1253-8. **2.** von Zur Muhlen *et al.* (2008) *J Clin Invest.* 118:1198-207. **3.** van Kasteren *et al*. (2009) *PNAS* 106(1):18-23. **4.** Serres *et al.* (2009) *J Neurosci* 29(15):4820-8. **5.** Hoyte *et al*. (2010) *J. Cereb. Blood Flow Metab*. E-pub. **6.** Perez-Balderas *et al* (2010) *ISMRM.*