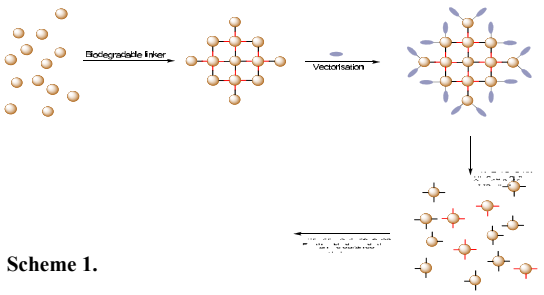
Multimeric iron oxide micro particles: novel high sensitivity and biodegradable MRI contrast agents.

F. Perez-Balderas[1], B. G. Davis[2], S. van Kasteren[2], A. Khrapichev[1], D. Anthony[3], and N. R. Sibson[1]
[1]CR-UK/MRC Gray Institute for Radiation Oncology and Biology, University of Oxford, Oxford, United Kingdom, [2]Chemistry Research Laboratory, University of Oxford, Oxford, United Kingdom, [3]Department of Pharmacology, Univerity of Oxford, Oxford, United Kingdom

Introduction: Due to their favourable properties (high magnetization, low toxicity) superparamagnetic iron oxide nanoparticles have been widely used for biomedical and biological applications such as MRI, hyperthermia, *in Vitro* bioseparation, sensing of different biomolecules and multimodal imaging. Due to their longer half life and better extravasation properties, which ensure that a large number of particles can reach their target, ultrasmall superparamagnetic particles of iron oxide (USPIO) have commonly been employed for MRI. However, for some applications the long half-life of such particles can be a limitation since this maintains a high background level of particles in the circulation and effectively reduces the contrast from specific deposition of the particles at the site of interest. When extravasation is not a requirement, larger particles have better contrast to noise ratios and are rapidly cleared from the circulation. Recently, microparticles of iron oxide (MPIO) have demonstrated superior capabilities for imaging of endovascular cellular events such as inflammation and activated platelet formation,[1, 2] and have been used to detect pathologies as multiple sclerosis, thrombosis, atherosclerosis and murine cerebral malaria in animal models.[1-5] Despite their successful use in animal models the polystyrene coated MPIO currently used (Dynal beads) suffer from a lack of biodegradability and potential toxicity that precludes their medical use.



Scheme 1.

Herein we report the synthesis of biodegradable multimeric iron oxide particles of *ca.* 1µm diameter. These multimeric particles are composed of several iron oxide nanoparticles (NPs) coupled through a protease labile peptide linker (Scheme 1). Subsequent vectorisation of the multimeric particles will allow targeting to specific molecules and enable disease detection. Degradation of the linker by a protease liberates the small size NPs that are easily cleared from the body.

Methods: Amino terminated nanoparticles (H_2N-NPs) were obtained by sequential treatment of a basic suspension of dextran coated iron oxide colloid with epichloridrin and ammonium hydroxide. These H_2N-NPs were modified by treatment with succinic anhydride to produce carboxylic acid terminated nanoparticles (HOOC-NPs) or with a peptide containing a peptidase recognition domain to produce peptide terminated nanoparticles (H_2N-peptide-NPs). Particle size was determined by dynamic light scattering (DLS) in a Malvern 3000 HS apparatus. Multimeric particles dissolved either in $NaHCO_3$ buffer pH 7.4 or citrate buffer pH 5.5 were treated with thrombin, cathepsin B or cathesin L at 37 ºC for 24 h.

Results: Reaction of HOOC-NPs (preactivated with EDC and sulfoNHS) with H_2N-peptide-NPs furnish the desired multimeric particles in high yield (tipically >95 %). By choosing the appropriate HOOC-NP/ H_2N-peptide-NP ratio it is possible to obtain multimeric particles with a size ranging from 600 nm to 2µm. These particles are spherical in shape when observed by wide field microscopy and show lower T2 relaxation times when compared with the commercially available Dynal beads. Using DLS it is possible to demonstrate that the particles remain unaltered over long periods of time (> 3 months).

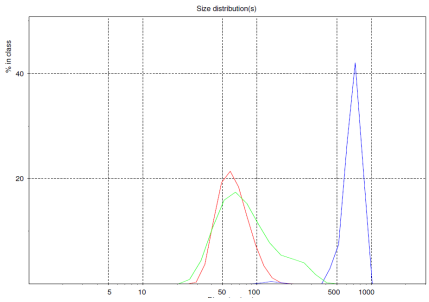


Figure 1. DLS size distribution graph of the starting NPs (red), multimeric particles (blue) and particles after thrombin treatement (green).

To demonstrate that the particles can be degraded to the original NPs they were treated with thrombin. DLS analysis showed that the size of the particles was reduced from ~600nm to 60nm corresponding closely to the size of the starting NPs (Figure 1). The primary sequestration sites of MPIO *in vivo* are macrophages in the liver and spleen, in which the cathepsins are the most abundant proteases. Therefore, we also treated the multimeric particles with the lysosomal proteases cathepsin B and L and similar results were obtained using DLS.

Conclusion: Biodegradable multimeric iron oxide microparticles were obtained by conjugation of iron oxide nanoparticles through peptides. These multimeric particles constitute an ideal platform for new highly sensitive and specific MRI contrast agents.

References:

[1] M. A. McAteer, N. R. Sibson, C. von zur Muhlen, J. E. Schneider, A. S. Lowe, N. Warrick, K. M. Channon, D. C. Anthony, R. P. Choudhury, *Nat Med* **2007**, *13*, 1253.
[2] C. von zur Muhlen, *The Journal of Clinical Investigation* **2008**, *118*, 1198.
[3] S. I. van Kasteren, S. J. Campbell, S. b. Serres, D. C. Anthony, N. R. Sibson, B. G. Davis, *Proceedings of the National Academy of Sciences* **2009**, *106*, 18.
[4] S. Serres, D. C. Anthony, Y. Jiang, K. A. Broom, S. J. Campbell, D. J. Tyler, S. I. van Kasteren, B. G. Davis, N. R. Sibson, *J. Neurosci.* **2009**, *29*, 4820.
[5] M. A. McAteer, J. E. Schneider, Z. A. Ali, N. Warrick, C. A. Bursill, C. von zur Muhlen, D. R. Greaves, S. Neubauer, K. M. Channon, R. P. Choudhury, *Arterioscler Thromb Vasc Biol* **2008**, *28*, 77.