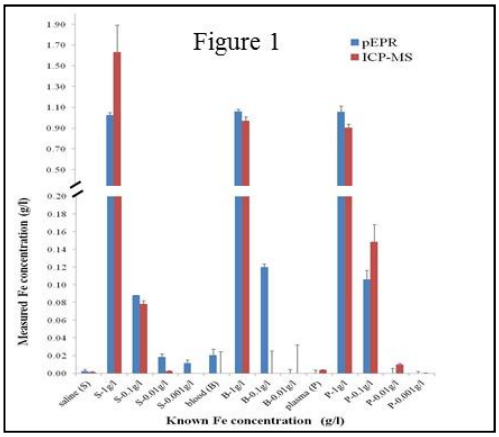
Pharmacokinetics and biodistribution of superparamagnetic nanoparticles using pEPR in the mouse: an in vivo, in vitro and ex vivo validation.

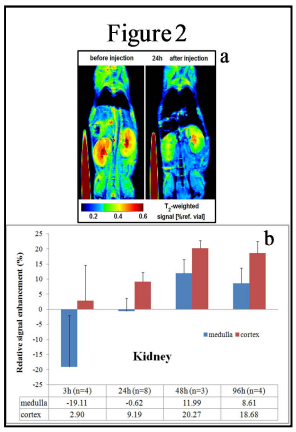
Oliviero Gobbo[1,2], Friedrich Wetterling[3], Kieran Crosbie-Staunton[2], Peter Vaes[4], Stephanie Teughels[4], Farouk Markos[5], Marek Radomski[1], Adriele Prina-Mello[2], and Yuri Volkov[2]

[1]School of Pharmacy and Pharmaceutical Sciences, Trinity College Dublin, Dublin, Ireland, [2]School of Medicine, Trinity College Dublin, Dublin, Ireland, [3]School of Engineering, Trinity College Dublin, Dublin, Ireland, [4]PEPRIC nv, Leuven, Belgium, [5]Physiology, University College Cork, Cork, Ireland

Purpose: It is anticipated that nanostructures will play a crucial role in medicine by serving as carriers for drugs and imaging agents that will bind to targets on injured tissue (1). It is therefore essential that we are able to detect these potential therapeutic agents. In our project, we are using a strategy based on the multifuntionalisation of superparamagnetic iron oxide nanoparticles (SPIO) combining diagnostic and therapeutic techniques against breast and pancreatic cancer (2). The aim of the present study was to assess and validate the particle electron paramagnetic resonance (pEPR) technique in a biodistribution study by means of *in vivo* MRI scans, and Inductively Coupled Plasma Mass Spectrometry (ICP-MS), as analytical gold standard technique. Here we present an alternative analytical method for detecting SPIO *in vitro* and *ex vivo* that is inexpensive and rapid (no sample preparation). It is based on electron paramagnetic resonance (3) and selectively measuring the magnetization of the SPIO. In pEPR a radio frequency excitation is applied at the resonant frequency and the received signal is a measure for the amount of SPIO present in the sample.

Materials and Methods: Quantitative validation of the pEPR technique was carried out by comparing pEPR and ICP-MS measurements using reference samples (in blood, plasma and saline) containing known quantities of SPIO. The concentrations are expressed in mg Fe/L and the difference measured between controls and samples represents the iron from SPIO. To assess the biodistribution of our DMSA coated iron oxide core nanoparticles (4), BALBc mice (25 ± 3 g) were anesthetized with isofluorane, positioned within a 7 T Bruker magnet and a baseline MRI was acquired. A second scan was performed at different time points (3, 24, 48, 96 hours) after the tail vein administration of either 100 µl of solution of SPIO (10 mg Fe/ml) or saline (vehicle control). Post scanning, mice were sacrificed and the organs harvested. The SPIO related signal change was evaluated on a same slice, which was located covering the organs of interest (heart, liver, spleen, lungs and kidneys). The baseline and SPIO images were co-registered using a control point method and an affine transformation in a script written in MATLAB®. The intensity of the signal in a given Region of Interest (ROI) was first normalized using a reference signal from a water tube located beside the animal. Relative Signal Enhancement (RSE) was defined as the relative change in normalized signal intensity (SI) pre and post SPIO injection [RSE (%) = 100 x (1-SI post/SI pre)]. A Turbo RARE T2-weighted sequence (TR/TE 4200/23.5 ms, FA 180°, FOV 6 cm, 5 averages, 35 slices, resolution: 306 µm/pixel) was used to record 2D images. The quantity of SPIO in the harvested organs was evaluated by pEPR. The concentration of SPIO is expressed in mg Fe/kg of organ and each organ is compared to a control across the different time points. The difference measured between organ controls and organ injected animals represents the iron amount from SPIO.





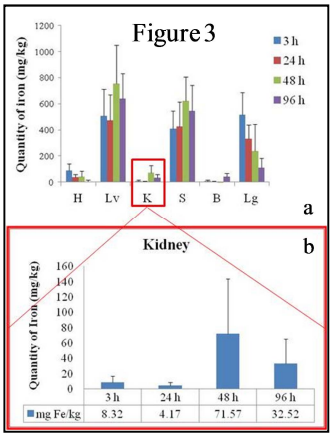


Figure 1. MNP detection in saline (S), blood (B) and plasma (P) by particle EPR and ICP-MS of samples containing known quantities of SPIO, (n=3).
Figure 2. a) Coronal T2-weighted images b) Example of intensity changes (RSA) over a 96 hour period.
Figure 3. a) and b) Iron assay by particle EPR in mg/kg of organ in the follow-up study after the harvest at 4 time points (H:heart, Lv:liver, K:kidneys, S:spleen, B:brain, Lg:lungs), mean ± sem, t-test *P < 0.05, (n=4).

Results: The quantity of inorganic iron measured by pEPR and ICP-MS perfectly matched the concentration of iron in the samples (Fig 1). The thresholds of iron detection for both techniques are at 0.1 g/L in blood and plasma and at 0.01 g/L in saline. In MRI the signal change corresponding to saline administration was constant through the organs at the different time points (data not shown). Following the injection of SPIO, signal intensity (SI) changes (Fig 3) were different for each organ. For the liver and spleen, the maximum SI changes (100 %) were reached after 3 hours post injection. For the lungs, SI changes could not be seen pre and post SPIO injection. For the heart, RSE decreased over the 96 hours. Finally, for the kidneys (Fig 2b), RSE reached a plateau for the cortex (+ 20 % at 48 h and + 18.68 % at 96 h) and for the medulla (+ 11.99 % at 48 h and + 8.61 % at 96 h). After harvesting the organs, pEPR iron assay (Fig 3a) showed high concentrations of SPIO in the liver, spleen and lungs and a small amount of iron was found in the heart and brain compared to controls. A decrease of the iron concentration in the lungs and the heart was recorded over the 96 hour period; whereas for the kidneys (Fig 3b), the concentration of iron increased slowly, reaching a peak 48 h post injection before declining at 96 h.

Discussion and conclusions: This study validates the use of a new analytical method of SPIO detection. We showed that a pEPR technique can be used to quantify the concentration of SPIO with the same sensitivity as the ICP-MS technique, in blood and plasma. This technique could, therefore, be used to assess the pharmacokinetics of SPIO. The distribution of SPIO *in vivo* seen in MRI was consistent with the concentration of iron measured by pEPR. From the results presented we can see that this consistency is particularly evident for the kidney, (as shown in Figs 2b and 3b) but could also be recorded in the liver, spleen, heart (Figs 2a and 3a). These preliminary data indicate that when studying the biodistribution and pharmacokinetics of SPIO, the pEPR technique is a good alternative and even has benefits such as sample preparation, selectivity and cost reduction compared with the ICP-MS technique.

References: 1) Kateb B., Neuroimage. 2011 Jan; 54, 24; 2) http://www.multifun-project.eu/; 3) Hubrich M, J. Magn. Reson. 1995, 114, 271-73; 4) Prina-Mello A., IEEE Trans Magn, 2012 (in press).