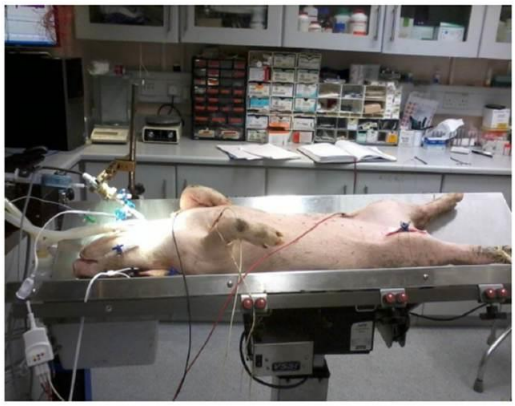
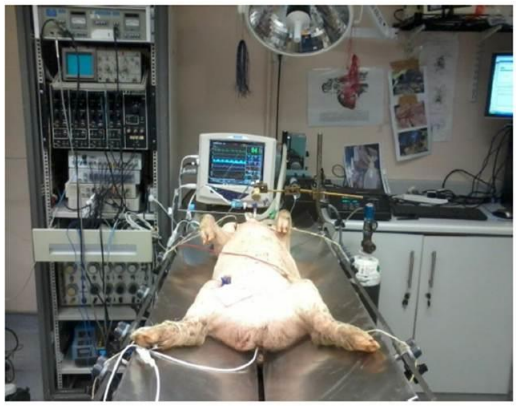
Benchmark study pEPR and ICP-MS

A benchmark study demonstrated that Pepric's pEPR method can beat the commonly accepted method of mass spectrometry (ICP-MS). The pEPR method was able to determine the pharmacokinetics of therapeutic magnetic particles.
In the experiment several Landrace piglets received an injection of magnetic nanoparticles.
Based on the tests with ICP-MS it was impossible to come to conclusive results.
With pEPR however we succeeded to determine the bloodclearance time of the particles.





The objective of the experiment was twofold. On the one hand, the particle was evaluated for the efficiency of the coating. The time during which the particle remains in the blood pool is crucial, the blood clearance time. It should be long enough to allow sufficient circulation enabling the specific binding of the particle with the target tissue. On the other hand the pEPR quantification of the particle content within the raw blood was compared to quantitative determination of the iron content of the sample through mass spectrometry, and benefits of pEPR were demonstrated.

At time t_0 a calibration blood sample was taken and a certain dose of particles was injected. Then blood samples were taken at several time points t_i and particle and iron content were measured. One expects a high level of particles when the particles were administered continuously decreasing with time.

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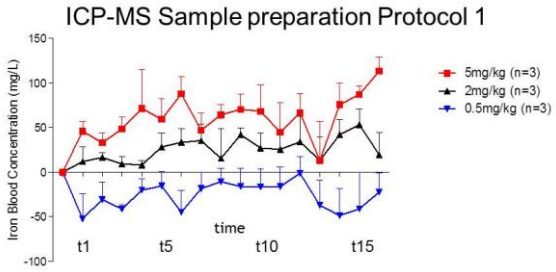
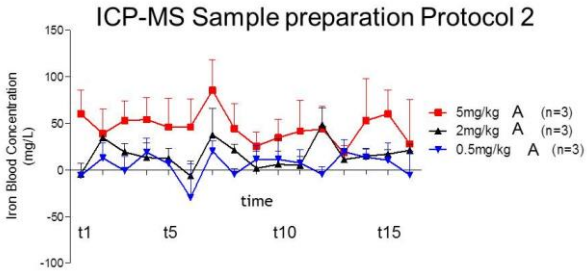
A first particle 'A' was injected in 3 different doses (5mg/kg, 2mg/kg and 0.5 mg/kg), and blood samples were taken. For the ICP-MS measurements, the blood samples were mineralized and strongly diluted. Two different dilution protocols were used on two fractions of the same samples and compared. To obtain the amount of iron of the particles, the amount of endogenous iron naturally present in the blood (measurement at t_0) was subtracted.

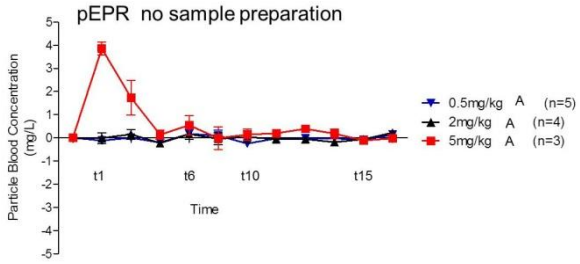




Conclusions

- ICP-MS is not selective for the iron of the particles. The subtracted level of endogenous iron is of the same order and even higher than the level of iron of the particles. This results in a rather inaccurate value of the particle concentration.
- A clear sampling error is shown caused by the manipulation and dilution of the samples although the blood samples were taken at the same time points and from the same piglets.
- The blood samples are destroyed and lost, therefore no further analysis is possible.

A third fraction of the same samples were analyzed with pEPR. The raw blood samples were used since pEPR does not require any sample preparation. Only heparin was added to avoid clotting during transport and storage. The pEPR signal amplitude is measured and converted to iron concentration to be able to compare with ICP-MS. The conversion was determined through calibration with a blood sample with a known particle concentration.



Conclusions

- pEPR is selective for the particles. The sample at t_0 was taken as a calibration, the background level is clearly below the signal of the particles. The clearance time was faster than anticipated and more samples had to be taken in between t_1 and t_5 for accurate determination of the blood clearance time.
- No sampling error could occur since the raw blood sample can be analyzed directly.
- pEPR is a non destructive method allowing further analysis of the same sample with other techniques if required.

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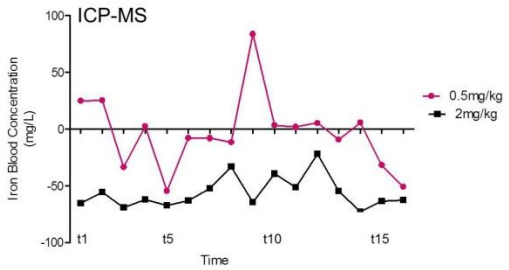
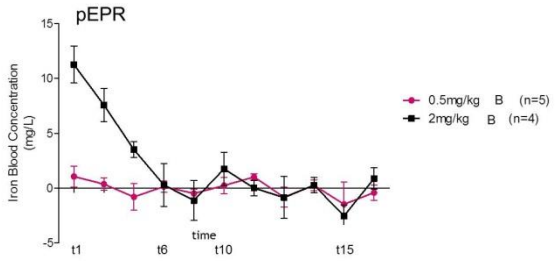
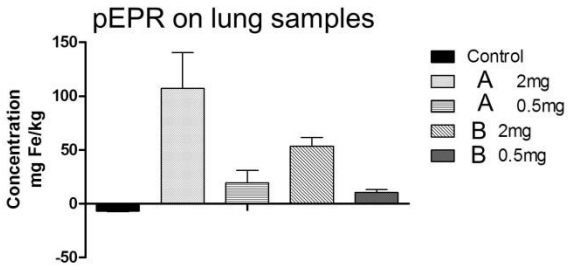
The bench mark experiment was repeated for a second particle 'B', injected in two different doses (2mg/kg and 0.5 mg/kg). Only one preparation protocol was used for ICP-MS and compared with the results obtained with pEPR. Also for this particle results remained inconclusive for ICP-MS and it was possible to determine the blood clearance time with pEPR.





The determination of the pharmacokinetics of the particles is still ongoing and first tissue samples of the piglets are analyzed. Several lung samples were taken after sacrificing the piglets at the final time point of the experiment. The samples were conserved in 10% Formalin for storage and transport to Pepric.
The amount of particles in the lung samples was determined quantitatively with pEPR. The results of the biodistribution will be compared with the MRI gray scale images and susceptibility measurements. One has to take into account that this is not possible for lung experiments since lungs are hardly visible on MRI.



Conclusions

- With ICP-MS no conclusive results were obtained, it was not possible to determine the blood clearance time of the particles.
- Based on the pEPR method the blood clearance time and biodistribution of the particles can be determined quantitatively allowing a clear understanding of the pharmacokinetics of the particles.

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