In-Vivo Ultra-High Resolution Imaging of Small Vessels using Improved Sensitivity and Long Circulation time of FeCo-Graphitic Carbon Shell Nanocrystals

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Introduction: FeCo graphitic carbon shell nanocrystals (FeCo/GC) [1] have been previously reported to have unprecedented high relaxivities with therapeutic properties. In this paper, we demonstrate how high sensitivity (relaxivity) combined with the relatively long circulation time (nano-scale size) can provide high-resolution imaging of the vasculature that can potentially visualize processes such as angiogenesis [2].

Methods: All MRI experiments were conducted at a 1.5 T GE whole body Signa Excite system (40 mT/s, 15000 mT/m/s). *(1) Material:* The FeCo/GC nanocrystals have been further refined to make them suitable for in-vivo applications. Uniform size makes it more favorable for enhancing blood circulation time and reducing r_2 / r_1 ratio which is desirable for highly sensitive positive contrast blood pool imaging. *(2) Signal Enhancement Comparison:* The expected signal enhancement vs. concentration for a 3D SPGR sequence (T_R = 33 ms, T_E = 4 ms, 30° flip angle) was calculated. Normal white rabbits were scanned with Combidex, 4 nm, 7 nm FeCo/GC injection amount targeting 0.2 mM blood pool concentration. The images were obtained 1 hr post-contrast injection. *(3) Aortic Stenosis:* A rabbit with ~100 % aortic stenosis was imaged with 7 nm FeCo/GC contrast injection targeting 0.2 mM blood pool concentration. *(4) High-Resolution Imaging:* 3D SPGR sequence with stack-of-spiral readout and spectral-spatial excitations was used to obtain a 78 x 78 x 500 um^3 spatial resolution. Small 1 inch surface coil was used to compensate for low SNR due to small voxel size. Normal white rabbits were injected with Combidex and 7 nm FeCo/GC targeting 1 mM and 0.2 mM concentrations respectively. Images were obtained immediately after contrast injections. Overall, a total of 10 rabbits were scanned.

Results: The improved material shows marked enhancement in r_1 (Table 1). The calculated signal enhancement predictions were successfully verified through aortic enhancement measurements. 7 nm FeCo/GC provides significantly higher signal enhancement (Fig.1c). With the 7 nm FeCo/GC injection, angiogenic collateral vessels formed around the aortic stenosis could be clearly visualized (Fig. 2). Using fast, high resolution imaging methods in combination with the contrast agent, 7 nm FeCo/GC provides enough contrast at a 10% of recommended Magnevist (Gd Complex) dosage while Combidex is unable to deliver such contrast even at 5 times the 7 nm FeCo/GC dosage.

Conclusion: FeCo/GC shows great potential to visualize micro-vasculature such as those involved in angiogenesis (< 100 um) at 10% of the recommended Magnevist dose.

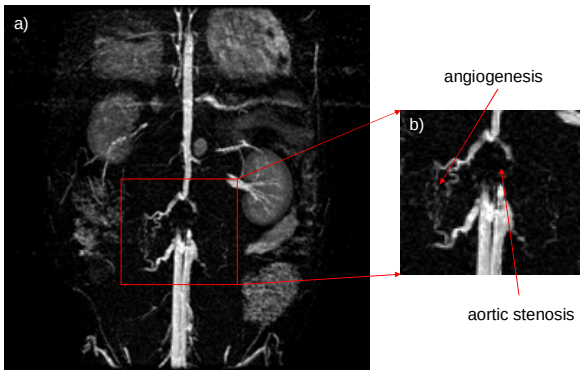
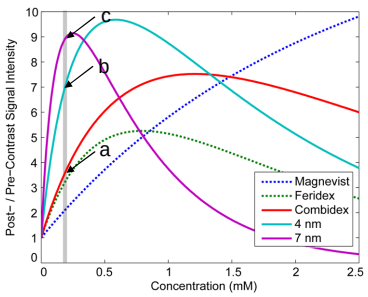


Figure 2: Rabbit aorta with a ~100% aortic stenosis. A FeCo/GC enhanced 3D SPGR maximum intensity projection (MIP) image (a) shows angiogenic collateral vessel formation around the stenosis. The newly formed micro-vasculature is clearly visible (b). Blood circulation downstream of the aortic stenosis can be explained by the connections provided by the angiogenic collateral vessels.

Sample	r_1 [$mM^{-1}s^{-1}$]	r_2 [$mM^{-1}s^{-1}$]	r_2 / r_1
Magnevist	5.4	5.5	1.0
Feridex	14	180	12.9
Combidex	15	97	6.5
4 nm FeCo/GC	45	160	3.6
7 nm FeCo/GC	97	400	4.1

Table 1: T_1 and T_2 relaxivities r_1, r_2, and r_2/r_1 ratios for commercial agents and PL-PEG-functionalized FeCo/GC nanocrystals at 1.5 T. The newly improved FeCo/GC nanocrystals show smaller r_2/r_1 ratios due to more uniform size distribution with clusters eliminated.



Enhancement

a) Combidex: ~3.8
b) 4 nm FeCo/GC: ~6.9
c) 7 nm FeCo/GC: ~9.5

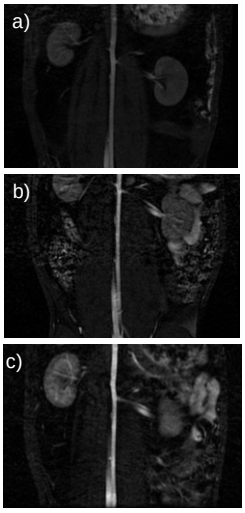


Figure 1: The left figure shows the calculated signal enhancement vs. concentration for commercial and developed agents. The right figures show verification of the predictions through rabbit aortic signal enhancement for Combidex (a), 4 nm (b) and 7 nm (c) FeCo/GC nanocrystals. 0.2 mM blood pool concentration was targeted. The difference in resulting signal enhancement can be clearly observed.

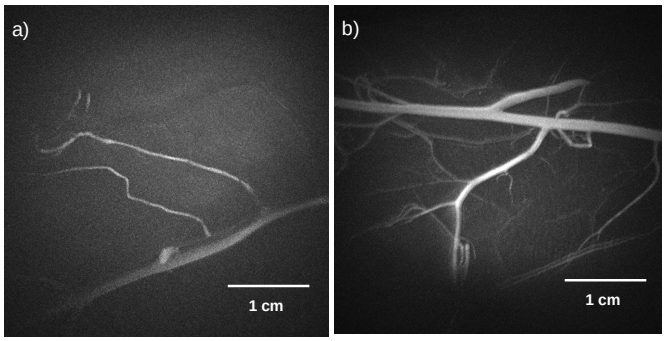


Figure 3: In-vivo high-resolution vasculature MIP image using a) ~1 mM blood pool concentration of Combidex and b) ~0.2 mM blood pool concentration of 7 nm FeCo/GC. Even with a concentration 5 times higher than that used for FeCo/GC, Combidex does not provide sufficient contrast for small vasculature (< 100 um) visualization.

References: [1] Seo et. al, Nature Mat. 2006. **5**(12): p. 971-6.
[2] Takeshita et al., J. Clin. Inv. 1994, 662-70.