Target-binding of Perfluoro-Carbon Nanoparticles alters Optimal Imaging Parameters using F-19 Molecular MRI: A Study using fast *in vitro* Screening and *in vivo* Tumor Models.

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Introduction

Patient stratification using molecular MRI of angiogenesis could fundamentally alter the standard of care and outcomes for anti-angiogenic therapy in combination with chemo- or radiation therapy [1]. Previously, $\alpha_v\beta_3$-integrin targeted nanoparticle (NP) emulsions [2] have been shown to detect angiogenesis and even to quantify the extent and distribution of angiogenesis for a number of tumor models in small animals. NP were visualized in pre- and post-injection ^{1}H-MRI via a Gd-chelate label [3, 4] or directly quantified using the ^{19}F-MR signal from the perfluoro-carbon core [5]. *In vivo* studies are currently performed to translate these promising results based on Perfluoro-Crown-Ether NP (PFCE), characterized by longer retention times, to a more clinically-relevant NP with a Perfluoro-Octyl-Bromide (PFOB) core. With a much more complex spectrum (7 ^{19}F resonance peaks, and multiple relaxation conditions), PFOB requires a thorough optimization of imaging parameters in order to be visualized *in vivo*. Furthermore, since the target binding of the NP strongly alters MR conditions, this optimization must be performed on target-bound NP, which is especially challenging for epitopes typically presenting concentrations below 100 mM_{19F} at the target. Herein, the *in vitro* optimization and *in vivo* detection of $\alpha_v\beta_3$-target-bound NP in the vasculature of Vx2-tumor bearing rabbits by ^{19}F-MRI on the PFOB-CF_2 resonances is demonstrated.

Methods

The study was performed on a 3T clinical whole-body scanner (Achieva, Philips Healthcare, NL) using a dual-tuned transmit/receive surface coil (7×12 cm) and a dual ^{19}F/^{1}H spectrometer system [6]. *In vitro*: Functionalized NP with a PFCE or PFOB core (20 vol%) were (a) targeted to acellular plasma clot surfaces (via avidin/biotin + antibody for fibrin-rich thrombi) and suspended in saline or (b) dissolved in agar at 150 mM (Figure 1 I-III). For optimization, a GRE sequence (matrix 128^2×15) was run without phase-encoding (1D-FT imaging), and frequency readout was oriented to differentiate different samples (resolution 1.9mm): pixel bandwidth (pBW) 150-500 Hz, flip angle 10-80°, 24 averages (3 minutes). A complex average of all profiles was used for SNR analysis. *In vivo*: Male New Zealand White rabbits (~2 kg, N=3) were implanted in the popliteal fossa of the left hind leg with 2-3 mm Vx-2 carcinoma tumors (National Cancer Institute, MD), which grew to 15 mm within 2 weeks. Imaging was performed 3h post-injection of 1.0 ml/kg of $\alpha_v\beta_3$-targeted NP with PFOB core. The rabbits were initially anesthetized with xylazine/ketamine *i.m.* and maintained with a 20 ml/h ketamine *i.v.* infusion. All animal care and protocols were in accordance with institutional guidelines. 3D gradient-echo sequences with concurrent dual-frequency RF/acquisition for ^{19}F and ^{1}H were used [6]: tuned to CF_2-resonance line group (5 lines, ±500 Hz range), voxel 5×5×4 mm^3, matrix 56×28, 15 slices, TR/TE=3.8/1.7 ms, flip angle α = 30°, pBW 500 Hz, 900 averages, scanning time 30 minutes. Standard high-resolution T1-weighted GRE images were also recorded for anatomical co-registration (resolution 0.55×0.55×4.0 mm^3, TR/TE=24/6.5 ms, α=35°, 6 min. scanning time).

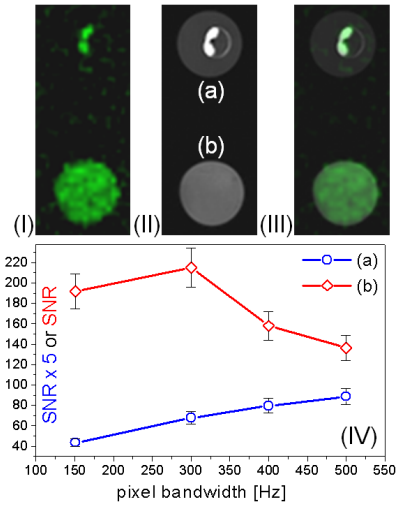

Figure 1: Optimization of MR parameters for optimal detection of target-bound NPs: ^{19}F (I), ^{1}H (II) and overlay (III) images of the arrangement, NPs bound to fibrin clot (a) or in agar (b). SNR measurement (IV) based on 1D-FT-imaging reveals altered parameter dependence (e.g. pixel bandwidth at α = 20°) of bound particles.

Results and Discussion

1D-FT imaging (using identical conditions as later imaging experiment except for phase-encoding gradients) was efficient for optimizing imaging parameters for NP at low concentration and at the target site. 16 different parameter combinations of pBW and flip angle were explored. Variation of the pBW at α = 20° (Figure 1-IV) revealed different behavior for free and target-bound NP. While free NP show an SNR-optimum at 300 Hz, bound particles favor large pBW. While noise is increasing at large pBW, signal increase at the corresponding short TE-values overcompensates in the bound NP case. Though the inherent mechanisms are under investigation, a potential explanation could be that bound NP exhibit enhanced relaxation and steady-state signal as compared to free NP. Using optimized parameter settings, bound $\alpha_v\beta_3$-targeted PFOB-NP could be visualized on Vx2 tumors in rabbits as shown in Figure 2. ^{19}F-signal could be observed at the tumor rim in all 3 rabbits, corresponding to areas with active angiogenesis, and also, as expected, in the epiphyseal heads and bone marrow [5].

Conclusion

^{19}F molecular MRI of target-bound nanoparticles requires parameter optimization on realistic conditions to detect low perfluoro-carbon concentrations *in vivo*. The applied *in vitro* model using fibrin clots is suitable for this purpose, but still different from soft tissue binding characteristics of a vascular target. Further improvement on *in vivo* ^{19}F signal from PFOB-NP, e.g. including all resonance lines and exploiting steady-state conditions, is currently being investigated.

References

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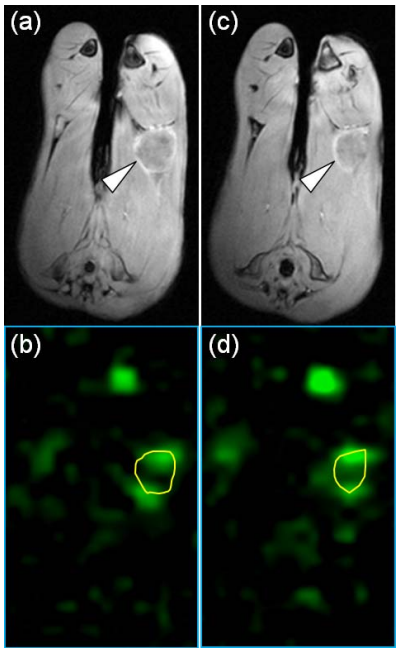

Figure 2: 3D molecular imaging of $\alpha_v\beta_3$-targeted NP on Vx2 tumor (arrow) in rabbits by ^{19}F MRI (b,d) from the PFOB core. 2 adjacent image planes out of 15 are shown. Simultaneous ^{19}F and ^{1}H acquisition was completed within 30 minutes. Anatomical co-registration is demonstrated by comparison to high-resolution T1-weighted ^{1}H GRE images (a,c).