PERFLUORO-15-CROWN-5-ETHER-LOADED HOLLOW MESOPOROUS SILICA SPHERES FOR 19F IN VIVO MRI

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TARGET AUDIENCE: Researchers interested in the approbation of the novel contrast agents for ^{19}F-MRI/ MRS.

PURPOSE: Para- and superparamagnetic contrast agents (CA) applied in ^{1}H MRI are detected indirectly by effecting the relaxation parameters of the surrounding water protons. Since CA relaxivities are not precisely known after administration, quantification is difficult. ^{19}F-based contrast agents are directly detected and enable quantification in ^{19}F-MRI and MRS. Due to its high NMR sensitivity (83% of ^{1}H) and negligible presence in tissue, ^{19}F offer great potential for *in vivo* quantification without any natural background signal. Perfluorocarbons such as perfluoro-15-crown-5-ether (PFCE), present with high ^{19}F load, excellent biocompatibility and a single NMR resonance, thus being attractive candidates for use in ^{19}F MRI. Mesoporous amorphous silica nanoparticles (MSNs) gain interest as drug carriers due to surface chemistry, flexible particle dimensions, high biocompatibility, in vivo degradation within several days, and systemic elimination by urinary excretion. However, MSNs enable only limited PFCE load due to the silica pore walls inside the particles causing volume exclusion effects. In this contribution, 250nm- and 1500nm-sized hollow mesoporous silica spheres (HMSS) are loaded with PFCE and evaluated as MRI CA *in vivo*.

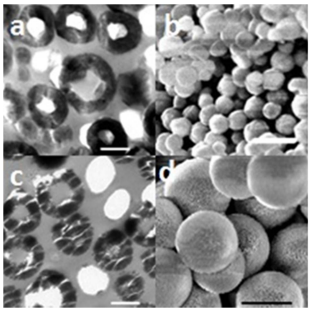

Figure 1. Microtomed TEM (a, c) and SEM (b, d) pictures of 250nm and 1500nm HMSS

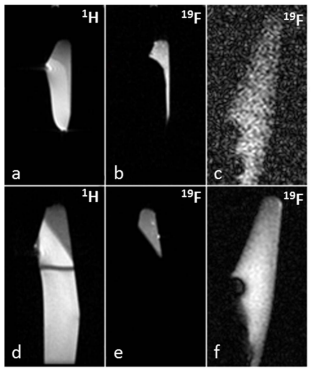

Figure 2. ^{1}H (a, d) and ^{19}F (b-f) pictures of the pellets (b, e) and dispersions (c, f) of 250nm (b, c) and 1500nm HMSS (e, f)

METHODS: The HMSS of 250nm and 1500nm-diameter were synthesized as described by Pochert et al[1]. For PFCE loading, the particles were dried in a Schlenk tube under vacuum for 4h at 100°C and cooled down. After PFCE was added, the system was frozen and evacuated again with subsequent heating at 160°C for 3 days. The ^{19}F-MRI of 250nm and 1500nm-sized PFCE-loaded HMSS in pellet and dispersed forms was performed with a FLASH sequence. Multiple inversion-recovery and spin echo experiments were performed for characterization of MR relaxation parameters. For quantification of the fluorine load, *in vitro* ^{19}F MRS of the particles was performed and compared to a reference probe of 500mg PFCE. All imaging parameters were kept constant throughout the experiments to allow quantification of the ^{19}F load by referencing to the reference probe. After MRI measurement the particles were dried and calcinated at 550°C for 6h to evaluate the exact mass of as used particles. In *in vivo* experiment 100µL of high-concentrated particles' dispersion (15mg/300µL) was injected via the tail vein of the mouse. ^{19}F *in vivo* imaging was performed using FLASH sequence. Additionally localized spectroscopy experiment was performed *in vivo*.

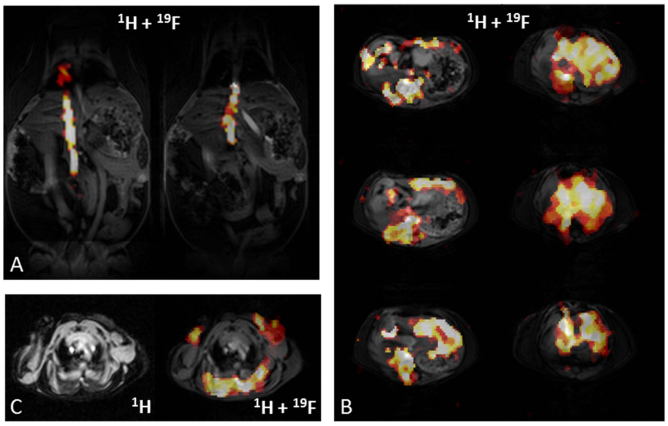

Figure 3: *In vivo* ^{1}H+ ^{19}F MRI following injection of 100µL of 250-nm-sized particles' dispersion via the tail vein. (A) ^{19}F signal could be directly detected in aorta. (B) ^{1}H axial slices through the liver with the overlapped ^{19}F signal. (C) Fat-suppressed ^{1}H image of the neck and ^{1}H+^{19}F image demonstrating the accumulation of the ^{19}F signal in the region of brown fat localization.

RESULTS: Microtomed transmission electron (TEM) pictures reveal the hollow interior of the particles and the mesoporous shell and its thickness (fig.1 a,c). Scanning electron (SEM) pictures (fig.1 b,d) provide detailed information of the particle dimensions. MRI images revealed an excellent fluorine signal for both 250nm- and 1500nm-sized PFCE-loaded HMSS in pellets (fig.2 b,e) and dispersions (fig.2 c,f). T_1 of 540ms and T_2 of 217ms were measured in a pellet of 250-nm sized HMSS (pure PFCE reveals T_1 of 650ms and T_2 of 563ms at 11.7T). Based on the MRS spectrums and respective FID amplitudes the factors relating the signal amount in a probe to the signal amount in the reference were calculated yielding values of 0.018 for 250nm-diameter HMSS and 0.029 for 1500nm-diameter HMSS. This is in a good agreement with the theoretically calculated loading capacity factors of 0.03 for 250nm HMSS and 0.07 for 1500nm HMSS, evaluated based on the respective particles' mass and size. Following the direct injection of 250nm-sized particles' dispersion, intense MR signal could be detected in the aorta (fig. 3A). Within 30 minutes ^{19}F MR signal of high intensity accumulated in the liver (fig. 3B) and, interestingly, in the brown fat (fig. 3C). *In vivo* ^{19}F spectrum localized in the liver reveals 2 peaks corresponding to isoflurane and 1 peak corresponding to PFCE-loaded HMSS (fig.4). Encapsulation of the PFCE causes a shift of the resonance frequency of about 2 ppm.

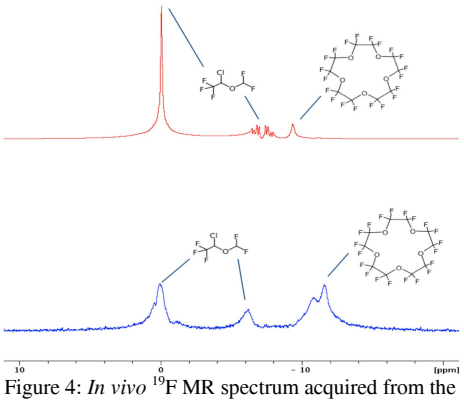

Figure 4: *In vivo* ^{19}F MR spectrum acquired from the liver (in blue) in comparison to the reference spectrum containing mixture of isoflurane and pure PFCE (red).

DISCUSSION & CONCLUSION: We could show that the hollow mesoporous silica nanoparticles can be effectively loaded with PFCE for generation of a good MRI signal. The local concentration quantification could be done using ^{19}F MR spectroscopy. Excellent ^{19}F MRI and MRS signal could be detected *in vivo*. Biodistribution of the PFCE-loaded HMSS should be further investigated. With additional surface functionalization the particles are promising for molecular imaging.

REFERENCES: (1) Pochert A, et al. Hollow mesoporous silica spheres as ^{19}F-MRI imaging agents. WMIC. 2013.