in vivo Molecular Imaging of Angiogenesis in Murine Urogenital Organs with Targeted Perfluorocarbon Nanoparticles and ^{19}F *ultrafast* Turbo Spectroscopic Imaging (F-*u*TSI)

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Introduction

Understanding the physiology of angiogenesis during the menstrual cycle and early stages of pregnancy is of vital importance in the diagnosis and treatment of endometriosis and endometrial cancer as well as in the identification of factors leading to implantation failure and endometrial dysfunction related infertility[1]. Experimental work where chick embryo chorioallantoic membrane was used as an *in vivo* model has shown indications of angiogenesis taking place during the early proliferative phase as well as the secretory phases of the menstrual cycle, yet, the exact timing and mechanisms of new blood vessel formation in the endometrium, especially during human menstrual cycle, are still unknown[2]. As a method that would enable *in situ* investigation and monitoring of the angiogenic processes we propose the use of a MR molecular imaging method that visualizes endothelial vascularization by targeting integrin receptors that are expressed during angiogenesis to link the cytoskeleton of the newly formed endothelial cells to the extracellular matrix[3]. The method employs Fluorine *ultrafast* Turbo Spectroscopic Imaging (F-*u*TSI)[4] and acquires the MR response induced by perfluorocarbon (PFC) nanoparticles that are functionalized with peptides containing the Arg-Gly-Asp (RGD) chain.

Materials and Methods

Experiments were conducted in a 3.0T Philips Achieva system (Philips Healthcare, Best, The Netherlands) that was equipped with a special design ^{1}H/^{19}F small animal coil[5]. Female Swiss nu/nu mice were injected with nanoparticles that are composed of 20% (w/v) perluforooctyl bromide (PFOB), a PFC compound that originally was developed and used as a blood substitute and CT contrast material, and 2% (w/v) surfactant in physiological salt. The surfactant comprised DSPC, DSPE-PEG-2000, cholesterol, Rhodamine-PE and DSPE-PEG2000-maleimide, of which the latter was used to link the RGD peptide to the nanoparticles. The contrast material was introduced in two intravenous doses of 150 μL, one delivered at about 20 hours and the other about 4 hours prior to scanning, in order to increase the probability of catching the proper stage of the menstrual cycle which is expected to last 4-6 days in mice[6], in a non-invasive manner. Each imaging cycle consisted of 32 echoes generated at the offset frequency corresponding to the CF_2 peak. The echo trains were run in planar *pseudo*-radial trajectories by phase encoding gradients operating in 3D[7]. The acquired k-space comprised 64x64x7 encoding points, covering a FOV of 64x64x21 mm^3, hence, with a voxel size of 1x1x3 mm^3. The acquisition was performed with 8 signal averages, resulting into a total scan time of approximately 45 minutes. Spatially resolved spectra obtained with F-uTSI were transformed into images by integrating each spectrum over a 2 kHz interval incorporating the CF_2 peak only. Underlying anatomical images were obtained using an Gradient Echo based fast imaging sequence acquiring a FOV of 64x64 mm^2, with a k-space matrix of 128x128 points, and 20 slices of 1 mm. Registration of ^{1}H and ^{19}F images was easily realized by using the DICOM geometric parameters, since the dual tuned imaging setup allowed both to be acquired without compromising the positioning of the subjects. In order to confirm the presence of PFOB particles in the organs, the liver, spleen and uterus of the mice were excised *post-mortem* and measured for ^{19}F signal with single voxel spectroscopy in the presence of a perfluoro-15-crown-5-ether reference (PFCE).

Results and Discussions

The signal acquired in preliminary experiments was observed to localize primarily in the liver and spleen in accordance to the known clearance pathway of PFC compounds. In 3 out of 5 subjects a third location, the urogenital organ of the mice, was observed to exhibit ^{19}F signal (Figure 1), most likely due to nanoparticle accumulation facilitated by the specific interaction between the integrin receptors and RGD ligand. NMR spectra obtained on excised organs also revealed presence of ^{19}F signal in the uterus, although very weak in comparison to the liver and spleen (Figure 2). Notwithstanding the promising results, further pathological investigation of the uterus and ovaries is of paramount importance to confirm the findings. The potential of using MR molecular imaging in combination with PFC nanoparticles in monitoring ovarian and endometrial angiogenesis would further be strengthened by conducting a study where two types of perfluorocarbon nanostructs, only one loaded with RGD, are used in combination with F-uTSI, which has the capability of distinguishing the signals of multiple PFC's[8]. Such a study would serve as a non-invasive verification of specific binding as well as angiogenic activity in the uterus.

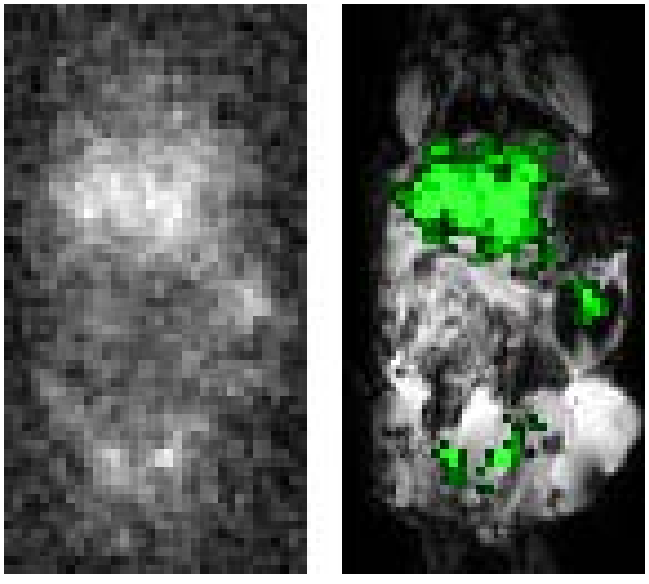

Figure 2 ^{19}F MR response obtained with intravenously injected RGD-PFOB nanoparticles (left). When overlaid on anatomic image, the Fluorine signal is observed to arise primarily from the liver and spleen as well as from the urogenital organ of the mouse (right).

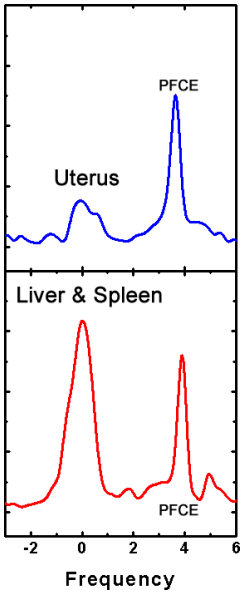

Figure 1 Single voxel spectra of excised organs compared against a PFCE reference.

References

1. T. Maruyama, Y. Yoshimura, Endocr. J., 55(5), p.795-810 (2008)
2. J.W.M. Maas, P.G. Groothuis, G.A.J. Dunselman, A.F.P.M de Goeij, H.A.J Struyker Boudier, J.L.H. Evers, Human Repr., 16 (8), p. 1557 (2001)
3. R.S. Robinson, K.J. Woad, A.J. Hammond, M. Laird, M.G. Hunter, G.E. Mann, Reproduction (2009)
4. M. Yildirim, J. Keupp, K. Nicolay, R. Lamerichs, Proc. Intl. Soc. Mag. Reson. Med. 15, p. 1249 (2007)
5. P. C. Mazurkewitz, C. Leussler, J. Keupp, T. Schaeffter, Proc. Intl. Soc. Mag. Reson. Med. 14, p. 2596 (2006)
6. A.K. Champlin, D.L. Dorr, A.H. Gates, Biol. Reprod., 8(4), p. 491-494 (1973)
7. X.B. Fan, J.N. River, A.S. Muresan, C. Popescu, M. Zamora, R.M. Culp, G.S. Karczmar, Phys. Med. Biol., 51(2) (2006)
8. R. Lamerichs, C. van Kammen, M. Yildirim, K. Nicolay, Proc. Intl. Soc. Mag. Reson. Med. 17, p. 616 (2009)