Combined in vivo confocal laser scanning microscopy and magnetic resonance imaging to study an *αvβ3*-integrin targeted nanoemulsion

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Introduction: Nanoparticles are widely used as drug delivery and molecular imaging platforms [1,2]. As drug delivery agents they have entered the clinic, as molecular imaging probes however, their application is primarily preclinical. The signal attenuation after i.v. injection of such agents is very variable throughout groups of subjects with the same pathological condition, hence there is a pressing need to study the underlying mechanisms and concepts. This study aims to increase the understanding of enhancement patterns post-injection (PI) of an αvβ3-integrin targeted nanoemulsion (fig. A) in tumors grown in an MRI compatible dorsal window chamber in mice. The nanoemulsions were labeled with both fluorescent lipids and iron oxide nanocrystals to allow their visualization with both confocal laser scanning microscopy (CLSM) and magnetic resonance imaging (MRI). With this setup, in vivo nanoparticle distribution patterns can be studied with CLSM at high resolution and clinically relevant macroscopic enhancement patterns can be visualized with MRI. To allow MRI of these small tumors we have developed a receive only Helmholz coil which accurately fits the window.

Methods and Results: Nanoemulsions were prepared by a solvent evaporation method and consisted of soybean oil droplets stabilized by a PEG2000-DSPE/DSPC/NIR664-PEG2000-DSPE lipid corona [3]. For relaxivity measurements, different amounts of 5 nm oleic acid coated iron oxide crystals were dispersed in the soybean oil core. The phosphate and iron contents were determined with inductively coupled plasma mass spectroscopy. By adjusting the ratio between soybean oil and phospholipids the size of the nanoemulsion was varied from 65 to 100 nm (with a polydispersity index below 0.15) as measured by dynamic light scattering. The ionic r_2 was measured as a function of nanoparticle size and Fe concentration in the soybean oil core on a 7 T animal scanner with a spin echo sequence. The r_2 was found to increase with nanoemulsion size and with Fe concentration in the nanoemulsion (fig. B, squares: 100 nm, circles: 65 nm).
For in vivo experiments, MR compatible window chambers were implanted on athymic nude mice. 1.5e6 HeLa cells were inoculated and two weeks after implantation tumors were used for experiments. Nanoemulsions of 100 ± 15 nm were used for in vivo experiments. The nanoemulsions were functionalized with αvβ3-integrin specific RGD peptides. RGD-functionalized or non-targeted control nanoemulsions were injected intravenously (i.v.) at a dose of 50 µmol of lipid per kg body weight and the tumors were imaged at different time points up to 24 hours PI. 2 MDa FITC-Dextran was injected to image the functional vasculature (in green) and cell nuclei were stained with Hoechst 33342 (depicted in blue), which was i.v. injected 20 minutes before imaging. In vivo CLSM demonstrated that RGD emulsions (in red) accumulated in the vicinity of the vasculature 10 minutes PI up to 24 hours PI (fig. E). Control emulsions (in red) marginally accumulated at early time points, but were found to be significantly extravasated from the vasculature 24 hours PI (fig. E). Agglomerates of RGD-emulsions associated with the vessel wall were observed in 3D reconstructions of CLSM data (fig. C). Interestingly, the RGD nanoemulsions were found in the vicinity of cell nuclei, which is indicative of cellular uptake (fig. D).
For initial testing of the Helmholz coil, a 4x4x0.4 cm^3 agarose phantom containing 0.1 mM Gd-DOTA was imaged with a RARE sequence (Rare factor: 4, TR/TE: 500/7.5 ms, flip angle: 90°, 6 averages, 500x390x390 μm^3, total scan time: 1m12s). Imaging of this phantom with the Helmholz coil and standard mouse- and rat-head coils demonstrated a 6 fold increase in SNR in case of the Helmholz coil (data not shown). In fig. F it can be observed that the Helmholz coil has highest sensitivity in the region where the tumors will be located (ROI 2 in fig. F). However, from calculations of the SNR in different parts of the field of view (FOV) (fig. F) it became apparent that signal outside the circular area needs to be suppressed to avoid folding artefacts when reducing the FOV. Applying 4 suppression slaps around ROI 2 resulted in virtually total crushing of signal outside of the circular high sensitivity area in case of this phantom (data not shown).

Discussion and Conclusion: The increase in ionic r_2 with nanoparticle size and iron oxide density in the soybean oil is most likely an effect of increased susceptibility effects and the exact mechanism is further investigated. The ultrahigh r_2 will be beneficial for detecting low concentration molecular targets. In vivo CLSM showed that differences in distribution patterns of targeted vs. non-targeted agents can be accurately observed up to at least 24 hours PI, at a (sub)cellular resolution. MRI with the Helmholz coil of the phantom demonstrated its potential for high resolution MRI of the small tumors in the window chambers. As the mice contain more water than the phantom used, we will have to carefully optimize the in vivo scanning protocol to avoid folding artifacts when reducing the FOV. Currently we are designing combination in vivo MRI CLSM studies of the same tumors. This experimental set up represents a powerful tool that will increase our understanding of the dynamics and subcellular distribution of the targeted agents and their effects on the clinically relevant MRI signal attenuation.

References: 1. W.J. Mulder et al., NMR Biomed., 2006; 2. M.M. Kaneda et al., Ann. Biomed. Eng., 2009; 3. P.A. Jarzyna et al., Biomat. 2009.