MRI *in vitro* setup for studying contrast agent effect in inhomogeneous environment

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Introduction

MR monitoring of local drug delivery resulted in the development of switchable MR contrast-agents to monitor release of nanocarrier content [1,2]. Soft tissues consist of several compartments, e.g. vascular, extracellular space and intracellular space. Nanocarriers and contrast agents will most of the times not distribute homogeneously within these compartments. This difference in distribution influences the capability for paramagnetic or superparamagnetic moieties to generate contrast. Currently, most in vitro studies concerning contrast agent release from nanocarriers are done in homogeneous gels, which especially for R2* relaxivity will not represent its expected behavior in tissue. We report on the design of a chitosan-coated alginate microsphere phantom to simulate the MR contrast properties of a commercially available paramagnetic contrast agent in a tumor-like environment.

Materials and Methods

Experimental setup: Alginate microspheres (Fig. 1), diameter 250 µm, were produced using the JetCutter technique [3] with a cross-linking solution containing 25 mM $CaCl_2$. After removal of the excess calcium ions, the, alginate-calcium microspheres were incubated in a 0.25% w/w chitosan solution (pH = 4, Acetate buffer, 100 mM $CaCl_2$) during 24h and washed with deionized water [4] in order to obtain chitosan-coated spheres impermeable to the gadolinium chelate. Five tubes were prepared containing a range of Gadobutrol (Gadovist) concentrations (from 0 to 1 mM) and a fixed volume of the coated microspheres (1mL). We assume that the Gadobutrol concentration in the bead-Gd compartment was diluted by a factor 4 as theoretically the volume in between the spheres in closest packing has a value of 1 - $\pi/3\sqrt{2}$ (≈26%) of the total volume.

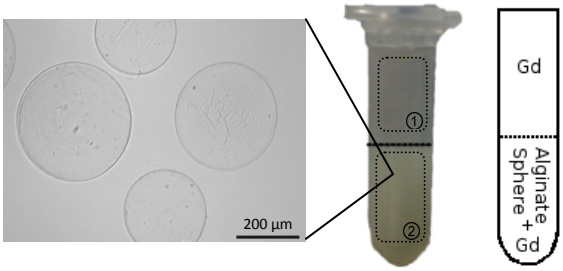

Fig. 1: Light microscopy of alginate microspheres (a) 2ml tube with Gadobutrol in alginate spheres and in free water.

Data acquisition: MR imaging was performed using a 1.5 T MR system (Philips, Best, The Netherlands). R1 relaxation rates were measured with a Look-Locker acquisition: TR/TE/α=1000ms/2.7ms/6°, with images acquired every 51 ms after inversion with a voxel size of 1×1×5mm^3. R2* values were obtained from a multi-echo spoiled gradient echo sequence: TR/TE_1/ΔTE/α =600ms/4ms/10ms/15°, 16 echoes, voxel size=1×1×3mm^3. Finally, R2 values were obtained from a multi spin-echo sequence: 16 echoes, TR/TE_1/ΔTE= 1000/30/30ms, resolution = 1×1×3 mm^3.

Data analysis: Relaxation rates were calculated using a Levenberg Marquardt fitting routine (IDL, Exelis, Boulder, CO, USA). ROIs of 140 to 180 pixels were drawn in the two compartments (Fig 1b.) to compare MR contrast agent relaxivities in a spatially inhomogeneous, environment (ROI) and in free water (ROI). Relaxation rates were obtained from linear regression of R1, R2 and R2* as a function of the volume average Gd concentration.

Results

Distribution of Gadobutrol through alginate microsphere beads without chitosan coating were characterized first:; no relaxivity differences (r1, r2 and r2*) were observed between the compartment containing beads and the compartment containing Gadobutrol in free water. With chitosan coating, alginate microspheres were impermeable to the contrast agent, which lead to a two-compartment phantom: one corresponding to free water (in between the spheres), the other corresponding to water enclosed in the spheres. In the presence of beads, no change of r1 relaxivity was observed. However, r2 and r2* did increase by a factor 5 and 7 (10.6→50.3 $s^{-1}mM^{-1}$ & 13.25→69.3 $s^{-1}mM^{-1}$), respectively, because of compartmentalization.

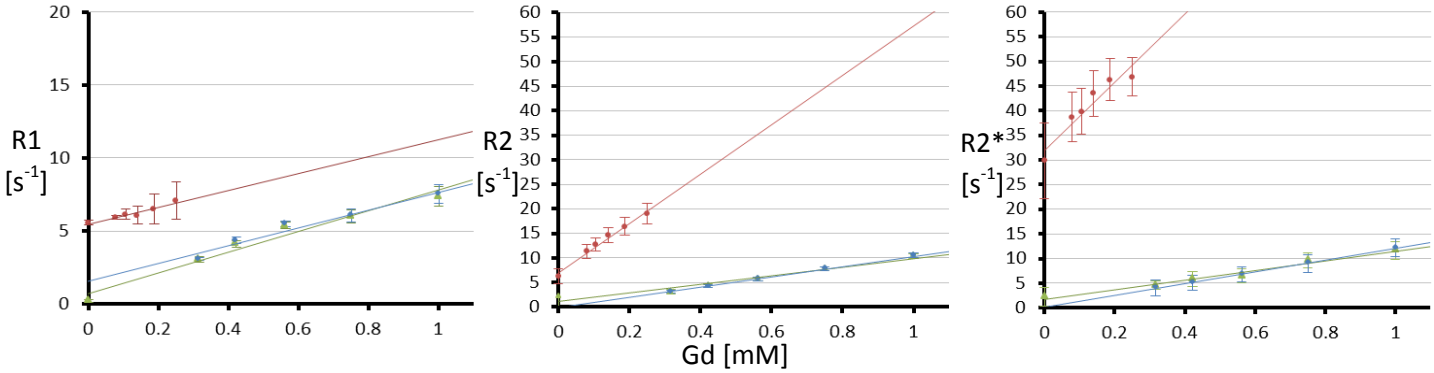

Fig. 2: Relaxation rates of Gadobutrol (Gadovist) in water (blue dots) above the beads (green dots) and in the presence of alginate beads (red dots)

Discussion

We presented a phantom that allows measuring the effect of MR contrast agents in an inhomogeneous compartmentalized environment. The beads represent (tumor-) tissue and the space in between mimics the vasculature representing the primary distribution volume of contrast agents. The construct can serve to mimic tumor tissue for contrast agent studies, especially those where R2 and R2* behavior is important. This effect was demonstrated for a typical gadolinium-based contrast agent (Gadobuterol) that showed a large change in relaxivity, depending on its environment. While compartmentalization in cells is known for iron oxide based contrast agents to be an important effect, which can easily be studied in cell pallets [5], not all nanocarriers studied are intended to be taken up by cells, but instead will initially distribute to the vasculature. Precise gadolinium concentration in the bead Gd compartment still remains to be confirmed by an independent modality such as ICPMS. We believe that these easily manufactured gel beads could be particularly useful for biodistribution studies on drug delivery systems. The system allows addressing questions related to quantification and detection thresholds in an *in vitro* experiment before an animal study is performed.

References

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Acknowledgment

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