

Evaluation of paraCEST detection threshold on human stem cells under physiological constraints

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Introduction:

Paramagnetic agent based chemical exchange saturation transfer (paraCEST) is capable of tracking several distinct cell populations, which is critical for non-invasively imaging brain tissue regeneration after stroke^{1,2,3}. Nevertheless, one key question for multi-color paraCEST application remains its detection threshold. To address this, we here aim to improve paraCEST scanning conditions for both Eu-HPDO3A (Eu) and Yb-HPDO3A (Yb) by considering both signal-to-noise ratio (SNR) and phantom temperature, then use such conditions for evaluating the detection threshold of paraCEST ex vivo.

In theory, CEST is normalized contrast that is independent of voxel size, but dependent on concentration. However, normalization should be affected by SNR since bulk magnetization appears in form of SNR, which is proportional to voxel size.

When the paraCEST agent is taken up into cells, it is normally localized to endosomes, which affects the imaging characteristics of the agents³. It is therefore essential that cells scanned as phantoms remain viable during the scanning procedure so that conditions will be comparable to in vivo. Cell viability was measured before and after scanning to ensure that this was the case.

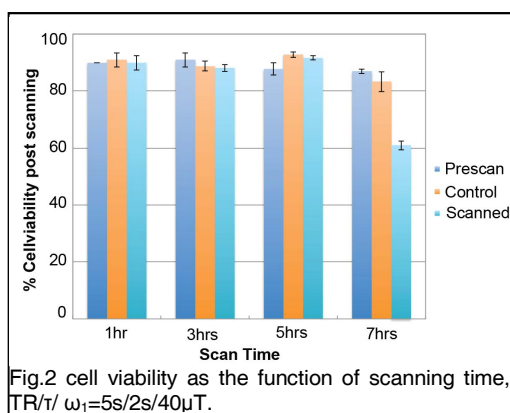


Fig.2 cell viability as the function of scanning time, TR/ τ / ω_1 =5s/2s/40 μ T.

CEST effect and sample temperature (Fig 1A &1C). A power over 40 uT decreased SNR to below 75 and led to either physiologically borderline temperatures (40°C) or above. To achieve a CEST contrast of at least 5%, a power of over 25 uT is needed (Fig. 1B &1D). As a consequence, specific absorption rate (SAR) is the limiting factor to further increase the CEST effect and applicable power is constrained between 25 - 40 uT by temperature. These imaging parameters did not affect the acute viability of cells after scanning up to a duration up to 5 hours. However, longer scanning times exhibited a significant reduction in viable cells (Fig. 2). Fig. 3 shows CEST effects as a function of voxel size when the concentration of paraCEST agents varies. As expected, CEST effects are not reduced as voxel size decreases. On the contrary, the amplitude of the CEST effect seems to increase as voxel size decreases. This is because the SNR of bulk magnetization acts as denominator in CEST quantification. As SNR decreases, the CEST effect falsely appears to increase. As a result, sufficiently high SNR is necessary for CEST quantification. Fig. 3 also indicates that both Eu and Yb have a detection threshold of 1mM in terms of concentration while the minimum feasible voxel size is about 0.016uL.

Conclusion: A major limiting factor in paraCEST imaging is the SAR, which increases sample temperature potentially above physiologically tolerable levels. Therefore, a long TR time is necessary to dissipate these adverse effects. We have here established scanning conditions that can sustain viable cells for scan duration of up to 5 hours. Using these scanning conditions, the detection threshold for paraCEST agents is ~1 mM in concentration and ~0.016uL in voxel size. Further work may include: 1) the assessment of any more subtle effects of SAR on cell biology; 2) addressing the ramification in CEST quantification caused by low SNR. One limitation of current work is the lack of theoretical prediction of CEST contrast for individual agents due to the shortage of literature on the physical properties of Eu and Yb. These developments will be essential to quantify paraCEST effect for tracking stem cells in regenerative medicine.

Refs. 1. Sztrihai, L et al, Monitoring Brain repair in Stroke using advanced MRI, Stroke, 2012 Nov. 2. 1-8.

2. Bible, E et al, ¹⁹F and diffusion MRI of transplanted human neuronal stem cells on rat brain. Biomaterials, 2012 Apr; 33(10):2858-71

3. Ferrauto, G et al, In vivo MRI visualization of different cell populations labeled with PARACEST agents. MRM, 2012, early view.

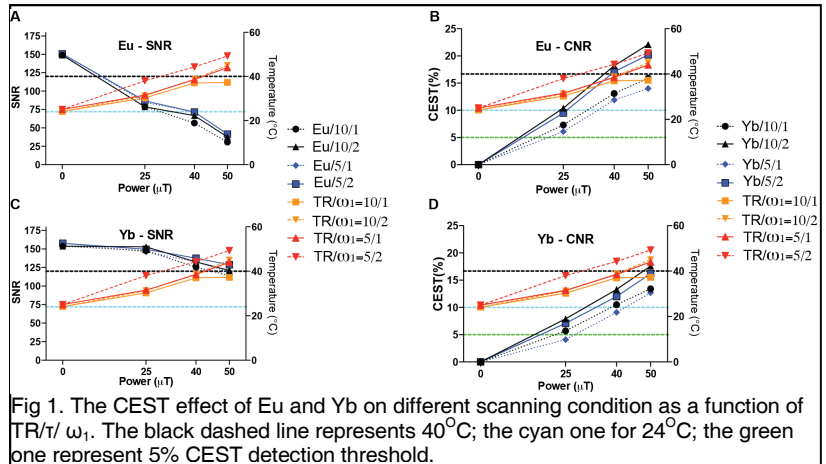


Fig 1. The CEST effect of Eu and Yb on different scanning condition as a function of TR/ τ / ω_1 . The black dashed line represents 40°C; the cyan one for 24°C; the green one represent 5% CEST detection threshold.

Materials and Methods:

Phantoms: Eu and Yb were dissolved in PBS solution, and transferred into eppendorfs with the concentrations of 10mM, 2mM, 0.5mM for both Eu and Yb; a blank control with PBS (Aldrich) was also prepared. The eppendorfs (Fisher) were fixed into a 30mL syringe filled with 2% agar (Aldrich)+ 0.07mM MnCl₂ (Aldrich). Human neural stem cells (STROc05 cell line, ReNeuron) were suspended in PBS at 50,000cells/ μ l (without paraCEST agent) and put into 0.2mL eppendorfs, which were fixed in 30mL syringes filled with 2% agar + 0.07mM MnCl₂. Samples were scanned for 1, 3, 5, and 7 hrs following by assessment of their viability using the Trypan Blue Exclusion test.

MRI experiments: Data were acquired on 9.4 T Varian VnmrJ using a 3.8 cm quad coil. The CEST contrast was accomplished by a squared pulse with various τ (duration) and ω_1 (amplitude) at the offset of interest followed by the spoil gradient GRE sequence with 45° flip angle and TE of 1.5 ms. In order to obtain the best CEST conditions, TR of 5s and 10s, τ of 1s and 2s, and ω_1 of 25 μ T, 40 μ T, and 50 μ T was tested on the phantom. The effects of voxel size on the CEST signal were determined using a fixed in plane resolution of 100 μ m x 100 μ m but varying slice thickness 1mm (0.032 uL), 0.5mm (0.016 uL), and 0.35mm (0.010 uL).

The temperature was recorded by optical temperature probe (Luxtron) on all MRI scanning.

Results & Discussion: Increasing the power for scanning decreased SNR and increased

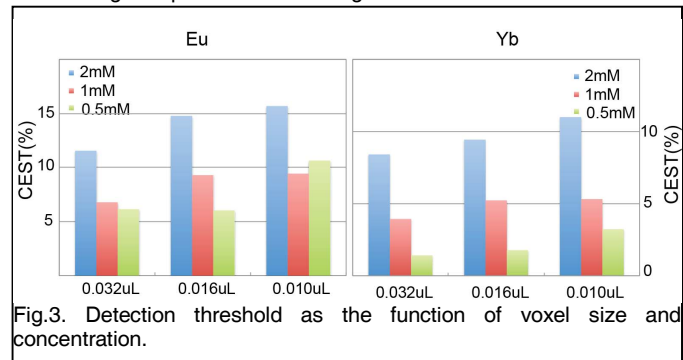


Fig.3. Detection threshold as the function of voxel size and concentration.