Magnetization transfer contrast MRI in GFP-tagged live bacteria

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Introduction– Green Fluorescent Protein (GFP) is a widely used molecular and gene expression marker which is non-toxic for both animals and bacteria. Published reports on *in vivo* MRI using GFP protein as a marker to label tumor (1) or stem cells (2) suggested that the labeling does not affect the gene expression. Recently, Magnetization Transfer Contrast (MTC) technique was used to detect GFP and was shown to produce protein-specific values that seemed to be concentration dependent (3). This provides a flexible, non-invasive *in vivo* molecular imaging system exclusively dependent on the concentration of the reporter GFP. Here, we compare wild-type and GFP-tagged *Pseudomonas aeruginosa* and *Escherichia coli* live bacteria using MTC MRI. This method was sensitive enough to distinguish between GFP-tagged and non-tagged bacteria at cell concentrations relevant to those used in animal infection models (4).

Materials and Methods– GFP-tagged *P. aeruginosa* (PA-GFP), (Figure 1), GFP-tagged *Escherichia coli* (EC-GFP) and non-fluorescent *P. aeruginosa* (PA) cells were grown in Luria broth overnight at 37°C. Bacteria were centrifuged, resuspended in PBS and diluted to final concentrations of 5 x 10^6 and 5 x 10^5 cells/ml. The latter concentration is equivalent to the *P. aeruginosa* inoculum used in a murine burn and infection model (1). 0.2 ml microfuge tubes were filled to the maximum capacity with the diluted cultures. We imaged the triple phantom in a 4.7 T horizontal bore magnet (20 cm bore diameter, Magnex Scientific, using a Bruker Avance console) The images were acquired in a 4.7 T horizontal magnet, 20 cm bore, Magnex Scientific, using a Bruker Avance console (Bruker Biospin, Billerica, MA) with a custom-built volume coil of 3 cm inner diameter and 10 cm active length. The main magnetic field (B_0) was shimmed and the RF filed (B_1) was calibrated. We acquired a RARE sequence (also known as Fast Spin Echo, FSE) with magnetization transfer (5-7). The imaging pulse sequence comprised a pre-saturation pulse at the designated offset frequency followed by a spin echo sequence with TE/TR=7.95/2000 msec. Images were recorded with a 128x128 matrix, Field of View = 3x3 cm, slice thickness = 3mm, and average = 1. Pre-saturation off-resonance pulses ranged from +/- 0.05 to +/- 0.4 kHz. Magnetization Transfer Ratios (MTR) in the form of *MTR = (Unsaturated - Saturated)* were calculated from the signal intensities of regions of interest (ROI) using Paravision software (Bruker Biospin, Billerica, MA).

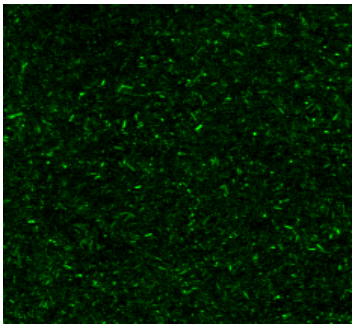

Figure 1. GFP-tagged *P. aeruginosa* cells viewed at 600x magnification.

Results– We compared MTC profiles of non-fluorescent *P. aeruginosa,* GFP-tagged *P. aeruginosa* and *E. coli* cells. Cells were visualized in 0.2 ml tubes filled to capacity with 5 x 10^5 cells/ml. The non-fluorescent *P. aeruginosa,* was chosen as a nonspecific control to compare against GFP-tagged bacteria, whereas *E. coli* was used as a specific control for the GFP to compare with both tagged and wild-type *P. aeruginosa.* The goal was to find the frequency at which there was the largest difference between GFP-tagged *P. aeruginosa* and *E. coli* cells and non-fluorescent *P. aeruginosa* (Figure 2). Samples were imaged first without and then with MTC. Nine MTC datasets were acquired from 0.05 to 0.4 kHz. From the images, the MTR was calculated and is shown in Figure 3. We found the largest difference between 0.2, 0.25 and 0.3 kHz (the peak difference is at 0.25 kHz) for EC-GFP and for PA-GFP with respect to PA. Figure 4 shows the calculated MTR values; we found a statistically significant difference between PA and EC-GFP (P<0.0001), but more importantly for our purposes an even greater difference between PA and PA-GFP, which was also statistically significant (P=0.00001).

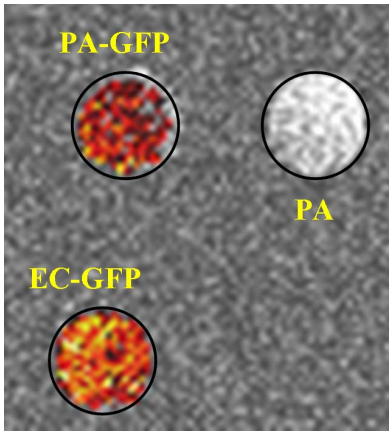

Figure 2. Pseudocolored pixel of EC-GFP, PA-GFP and PA at 0.25 kHz offset.

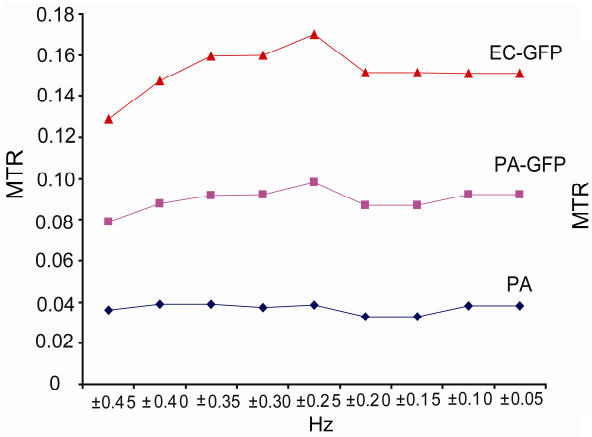

Figure 3. Region based MTR calculations for the different frequency offsets for PA, PA-GFP and EC-GFP from 0.05 to 0.45 kHz.

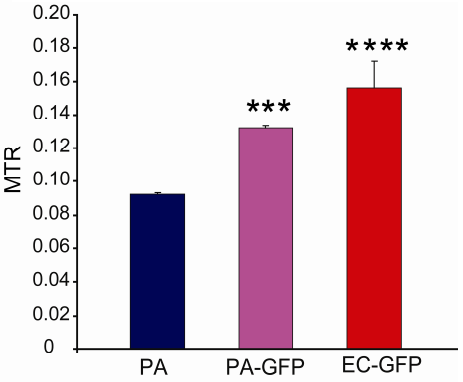

Figure 4. MTR (Mean +/- SE) of PA, PA-GFP and EC-GFP, P-values (***) <0.0001 and P-values (****) <0.00001

Discussion– Our results confirm the hypothesis that we can detect GFP-tagged live bacteria using Magnetization transfer contrast MRI. Our data suggest that GFP can be used to track bacterial proliferation and gene expression *in vivo* in animal models using a flexible, non-invasive technique. Furthermore, this *in vivo,* MRI molecular imaging system can detect varying levels of the GFP reporter, further establishing its utility for studying host-bacterial interactions. Our experience with assessing GFP-tagged *P. aeruginosa* in a murine burn and infection model to establish this methodology *in vivo* has been successful. The significance of this method is that it can be used to visualize bacterial infections *in vivo* in real time without being restricted to the use of transparent tissue necessary for optical imaging. This method provides a valuable, non-invasive imaging tool to study the impact of novel antibacterial therapeutics on bacterial proliferation and perhaps viability within the host system. Furthermore, the expression of relevant bacterial genes can be monitored during infection by expressing GFP under the control of appropriate bacterial promoters.

References

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