Magnetization Transfer Contrast MRI detects *Pseudomonas aeruginosa* bacterial infection *bacterial infection* a mouse burn model

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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. Magnetization Transfer Contrast (MTC) MRI has been previously utilized to distinguish intrinsic macromolecule concentration changes. Published reports on *in vivo* MRI studies 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, an MTC technique was used to detect GFP and was shown to produce protein-specific values that seemed to be concentration dependent (3). The use of an extrinsic protein marker provides an added flexibility. The main advantages of detecting GFP with MTC MRI over other MRI based reporters includes that there are multiple GFP mouse lines available and it poses no toxicity. This provides a flexible, non-invasive *in vivo* molecular imaging system exclusively dependent on the concentration of the reporter GFP. We report an *in vivo* study of GFP-tagged MTC MRI in a Burn mouse model infected with *Pseudomonas aeruginosa.* The goal of this study was to visualize bacterial infections *in vivo* in real time, and to study the impact of novel therapeutics on bacterial proliferation and viability within the host system (4).

Materials and Methods– GFP-tagged *P. aeruginosa* (PA-GFP) cells were grown in Luria broth overnight at 37°C. Bacteria were centrifuged, resuspended in PBS and diluted to final concentrations of 5×10^5 cells/ml. That concentration was used as inoculum in a murine burn and infection model (1). Six weeks old CD-1 mice were anesthetized and a leg burn injury of 5% total burn surface area was produced on the right thigh muscle. Mice were randomized into one experimental and control groups (N=6 per group). The experimental group consisted of burned mice GFP-tagged *P. aeruginosa* infected and the control group consisted of burn mice *P. aeruginosa* infected. The mice were imaged 12 hours post-burn and infection. During MRI, mice were kept anesthetized with a mixture of isoflurane and maintained at 37° C. Imaging was performed in a 4.7 T horizontal magnet (20 cm bore, Bruker Avance console) using a custom-built volume coil (3 cm inner diameter, 10 cm active length). The main magnetic field (B_0) was shimmed and the RF filed (B_1) was calibrated. We acquired images using a RARE sequence (also known as Fast Spin Echo, FSE) with magnetization transfer (5-7). The imaging pulse sequence included 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).

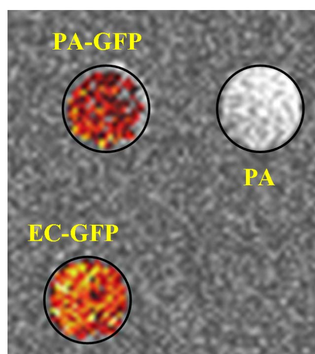

Figure 1. Pseudocolored pixel of EC-GFP, PA-GFP and PA at 0.25 kHz offset.

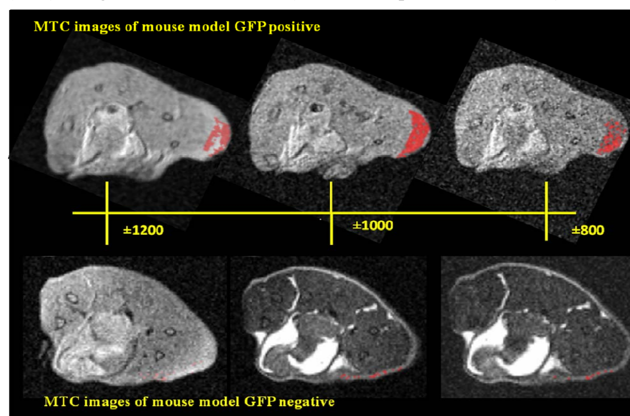

Figure 2. Pseudocolored pixel of GFP positive (upper) and GFP negative (lower) mouse MR images at 1 kHz offset.

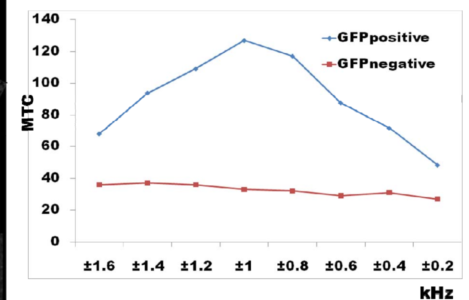

Figure 3. Region based MTR calculations for the different frequency offsets for GFP positive and GFP negative mouse model from 0.2 to 1.6 kHz.

Results– We compared MTC profiles of non-GFP fluorescent *P. aeruginosa* control mice and GFP-tagged *P. aeruginosa* infected mice. Figure 1 shows results from our *in vivo* PA14-GFP cell experiments. The goal was to find the frequency with the largest difference between GFP-tagged and non-tagged cells infected with *P. aeruginosa*. Figure 2 shows results from our experiments in mice. The two groups (GFP-positive and GFP-negative) were imaged first without and then with MTC. Nine MTC datasets were acquired from 0.2 to 1.6 kHz. From the images, the MTR was calculated and is shown in Figure 3. We found the largest difference between 0.8, 1 and 1.2 kHz, the peak difference being at 1 kHz, for the GFP-positive with respect to the GFP-negative mice.

Discussion– Our preliminary results on cells confirmed the hypothesis that we can detect GFP-tagged *P. aeruginosa* using MTC MRI. Our data suggest that GFP can be used to track bacterial proliferation and potentially gene expression *in vivo* in animal models using a flexible, non-invasive technique. To this end, we performed *in vivo* MRI molecular imaging using GFP-tagged *P. aeruginosa* in burn mice and detected the GFP reporter, establishing its utility for studying host-pathogen interactions. Our experience with assessing GFP-tagged *P. aeruginosa* in a murine burn trauma 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 will provide a valuable tool to study the impact of novel antibacterial therapeutics on bacterial proliferation and viability within a live 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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