

## A Gene Reporter System for the Detection of Cellular LacZ Expression MRI

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### Introduction

The purpose of this study was to evaluate the use of a histochemical substrate, commonly used for the *in vitro* detection of marker gene expression, as a substrate for the *in vivo* detection of  $\beta$ -gal expression in bone marrow cells (BMCs). Contrast agents have been extensively used for MR cell tracking studies but in many cases their use has been limited to following either the initial delivery of cells or to track slowly symmetrically, dividing cells(1). It may not always be possible to avoid rapid cell turnover or loss in many therapeutically relevant cases. For example following myeloablation and bone marrow transplantation, a small subset of BMCs called hematopoietic stem cells (HSCs) will respond to replenish the depleted bone marrow and restore homeostasis by rapid cell division. In order to track HSCs and their progeny *in vivo*, a genetic approach would be a valuable tool to follow this dynamic cell population. In this study we determine whether the widely established gene marker,  $\beta$ -galactosidase ( $\beta$ -gal), could be detected by MRI following exposure to S-gal and ferric ammonium citrate (FAC) *in vitro* and *in vivo*.

### Experimental

BMCs were harvested from C57 and ROSA26 mice and re-suspended with 1 mg/ml S-Gal (Sigma) and 0.5 mg/ml FAC (Sigma) in 1ml PBS, 0.5 mg/ml FAC in 1 ml PBS or 1 ml PBS in microcentrifuge tubes and incubated at 37°C for 2hrs. BMCs were then washed twice with PBS to remove free S-Gal and FAC and filtered through FACS tubes with cell strainer caps to remove large aggregations of S-Gal and cells. Finally, BMCs were counted, assayed for viability by Trypan blue exclusion test (n=5), and re-suspended at a concentration of  $8 \times 10^7$  cells/ml in 100  $\mu$ l PBS. Cell phantoms were made by adding 100 $\mu$ l 2% Agarose to the labeled BMCs and injecting the solution into 100  $\mu$ l capillary tubes to keep cells stationary during imaging (n=5). Phantoms with a final cell concentration of  $4 \times 10^7$  cells/ml were placed in a water filled FACS tube to minimize susceptibility artifacts at the edges of the samples. Phantoms were kept on ice and imaged with a  $T_2^*$  weighted FLASH gradient echo sequence (TR=0.5s; TEs=4,8,12,16,20,30,40,60,80,100ms, FOV:2.8 $\times$ 2.8cm<sup>2</sup>, Matrix size:256 $\times$ 256, NEX=2, BW=60 kHz, thick=1mm) at 4.7, 11.1, and 17.6. ImageJ software (NIH) was used to quantify  $T_2^*$  between field strengths of 4.7-17.6T. Data are presented as the mean $\pm$ SD of measurements.

Subsequently,  $0.5 \times 10^6$  S-Gal/FAC labeled ROSA26 and C57BL6 BMCs in 40  $\mu$ l PBS were injected into C57 muscles (left and right legs respectively). The animals were anesthetized using a mix of 2% Isoflurane in O<sub>2</sub>. The hindlimbs were placed inside a 1cm solenoid coil or loop-gap coil and imaged with a 3D-GE (TR=100ms, TE=5 ms, BW=100kHz, Flip=30°, FOV=1.45 $\times$ 1.20 $\times$ 2.40cm<sup>3</sup>, matrix=384 $\times$ 192 $\times$ 64) scan sequence at 4.7&11.1T.

### Results and Discussion

Trypan blue exclusion test indicate that labeling BMCs with S-gal/FAC has minimal effect on cell viability (Fig1A). BMC phantom data shows a reduction in  $T_2^*$  relaxation due to the specific reaction between S-gal and  $\beta$ -gal and that this  $T_2^*$  effect is markedly increased with increasing magnetic field strengths (Fig1B). The *in vivo* detection of S-gal/FAC labeled ROSA26 BMC was dramatically increased compared to labeled C57BL6 BMCs, as indicated by the much larger  $T_2^*$ -effect generated by the  $\beta$ -gal expressing BMCs in the left TA. *In vivo*  $T_2^*$ -contrast from the labeled cells was also enhanced with increasing magnetic fields.

### Conclusions

S-gal reacts with  $\beta$ -gal to produce a dark iron-rich precipitate that can be detected simultaneously by MRI and histology. We found that S-gal labeling reduced  $T_2^*$  relaxation time for  $\beta$ -gal expressing BMCs more than control cells and the change in  $T_2^*$  was greatly enhanced with increasing magnetic field strengths. The dramatic decrease in  $T_2^*$  relaxation time led to increased sensitivity and increased detection capabilities of transplanted BMCs *in vivo* at high magnetic fields.

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### References

[1] Walczak P et al. *Magn Reson Med* 2007;58(2):261-269.

