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Comparing the sensitivity and resolution of VivoTrax and VivoTrax+

Introduction

VivoTrax™ is a super-paramagnetic iron oxide (SPIO) tracer commonly used in Magnetic Particle Imaging (MPI). Although it has found wide application in MPI studies ranging from cell labeling[1] to neuroimaging[2], the overall sensitivity of the tracer is limited because most of the iron oxide nanoparticles in the sample do not contribute to the MPI signal[3,4]. Resovist is comprised of small 5 nm iron oxide nanocrystals, and a small percentage of the nanocrystals are closely bound together into clusters that magnetically interact to produce a signal. The remaining iron oxide nanocrystals, which form the majority of the sample, are magnetically non-interacting and do not produce a significant MPI signal. Magnetic Insight recently released VivoTrax+™, a filtered form of VivoTrax™ that increases the fraction of the larger clusters containing interacting cores, which improves MPI performance. This study directly compares the two agents by assessing sensitivity, cell labeling efficiency, and *in vivo* imaging.

Methods

Cell Labeling

A2058 human melanoma cancer cells were cultured at 37 °C in complete Dulbecco's Modified Eagle's Medium (Thermo Fisher Scientific, Waltham, Massachusetts) until 90% confluent. VivoTrax+ and VivoTrax (200µg Fe/mL) were added to separate cultures with protamine sulfate (0.24 mg/mL) and heparin (8 USP units/mL) as transfection agents. After overnight incubation, cells were washed 3 times with phosphate-buffered saline (PBS). Cell counting and viability was determined using the trypan blue exclusion assay (Countess Automated Cell Counter; Invitrogen). Perls Prussian Blue (PPB) staining was then performed to assess iron labeling. Iron content per cell was determined by imaging samples containing 1.0 x 10^6 labeled cells for each agent (2D, 3.0 T/m gradient, 22 mT (X) and 26 mT (Z) drive) and dividing the measured iron content (determined using a calibration line) by the number of cells.

MPI Relaxometry

MPI relaxometry was performed on triplicate samples containing 3 µL (16.5 µg Fe) VivoTrax+ or VivoTrax using the Relax™ module equipped on the Momentum™ scanner (Magnetic Insight Inc.). Relaxometry curves were analyzed using Prism software (9.3.0, GraphPad Inc.) for peak signal and full width half maximum (FWHM) values.

In Vitro Image Acquisition

Samples containing 62.5K, 31.3K, 15.6K, 7.8K, and 3.9K (K = 1 X 10^3) VivoTrax+ or VivoTrax labeled cells suspended in ~250 µL PBS were prepared. Projection images were acquired in 2D with a 3.0 T/m selection field gradient and drive field strengths of 22 mT and 26 mT in the X and Z axes, respectively. These 2D images took ~2 minutes to acquire for a 12 x 6 cm field of view (FOV).

In Vivo Image Acquisition

In vivo imaging was performed on nude mice 24 hours post intravenous (IV) injections of 40 µL (220 µg Fe) VivoTrax+ (n = 3) and VivoTrax (n = 3). Prior to imaging, mice were fasted for 12 hours with only water, a laxative, and corn bedding in their cage to reduce gastrointestinal signal. Mice were anesthetized with 2% isoflurane and maintained with 1% isoflurane during imaging. The same image parameters were used for 3D imaging, which combined 35 projections (~30 min).

Results

Cell Labeling
VivoTrax+ had a higher labeling efficiency than VivoTrax, with 7.5 pg Fe/cell compared to 4.2 pg Fe/cell. Cell viability was high for both agents, with 91% and 92% for VivoTrax and VivoTrax+, respectively (Figure 1).

MPI Relaxometry
MPI relaxometry showed significantly higher signal (~2.4x) for VivoTrax+ compared to VivoTrax (111.8 vs. 46.6) (Figure 2A). Resolution was also improved with VivoTrax+ by ~25% (VivoTrax – 9.9 mT, VivoTrax+ – 7.9 mT) (Figure 2B).

In Vitro Image Acquisition
VivoTrax+ enabled imaging of fewer cells compared to VivoTrax. As few as 15.6K VivoTrax+ labeled cells (5.3 ng Fe) were detected with this imaging protocol (Figure 3A) compared to 62.5K VivoTrax labeled cells (25 ng Fe), ~4x fewer (Figure 3B). Overall cell detection was improved for VivoTrax+ labeled cells.

In Vivo Image Acquisition
MPI images were acquired of six nude mice following IV injection of VivoTrax (n = 3) or VivoTrax+ (n = 3). MPI signal was observed in the mouse liver 24 hours post-injection, a result of uptake of iron by phagocytic Kupffer cells. Significantly more signal was detected in the livers of mice injected with VivoTrax+ compared to VivoTrax, demonstrating improved *in vivo* sensitivity (Figure 4).

Conclusion

This study demonstrates the improved performance of VivoTrax+ and its potential use for cell tracking applications. VivoTrax+ had a higher labeling efficiency compared to VivoTrax, using transfection agents as a labeling strategy to enhance cellular uptake. VivoTrax+ demonstrated higher cellular sensitivity, enabling imaging of fewer labeled cells than VivoTrax. Additionally, VivoTrax+ demonstrated higher sensitivity in vivo, with significantly more MPI signal detected in the livers of nude mice 24 hours post IV injection of the tracer.

References

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3. Gleich B, Weizenecker J. Tomographic imaging using the nonlinear response of magnetic particles. Nature; **435**, 1212 (2005)
4. Eberbeck D, Wiekhorst F, Wagner S, Trahms L. How the size distribution of magnetic nanoparticles determines their magnetic particle imaging performance. Applied Physics Letters; **98**, 182502 (2011)

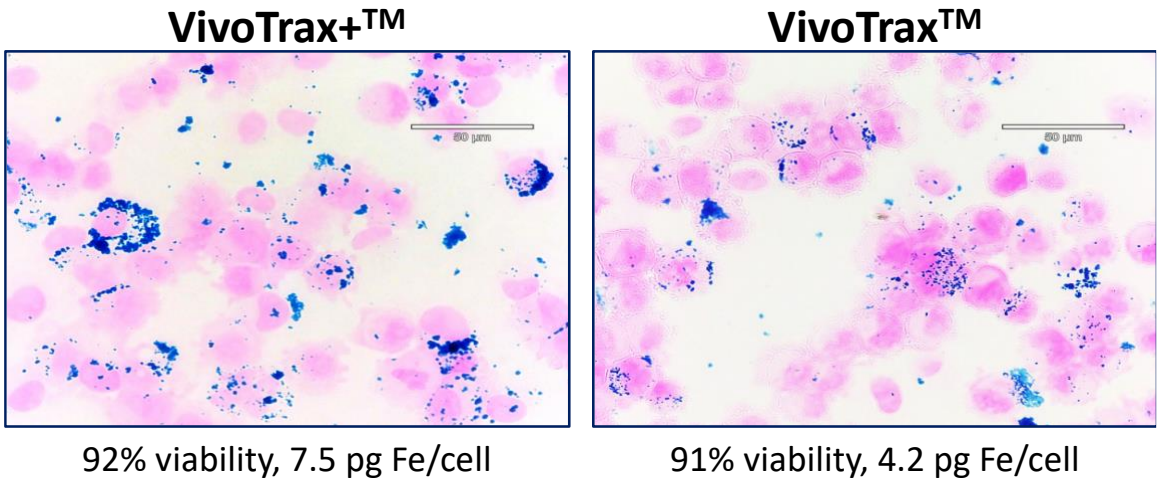

Figure 1: VivoTrax+ labels cells more efficiently than VivoTrax, with 7.5 pg Fe/cell compared to 4.2 pg Fe/cell. Cell viability was not affected by labeling and was high for both agents (VivoTrax+ – 92%, VivoTrax – 91%).

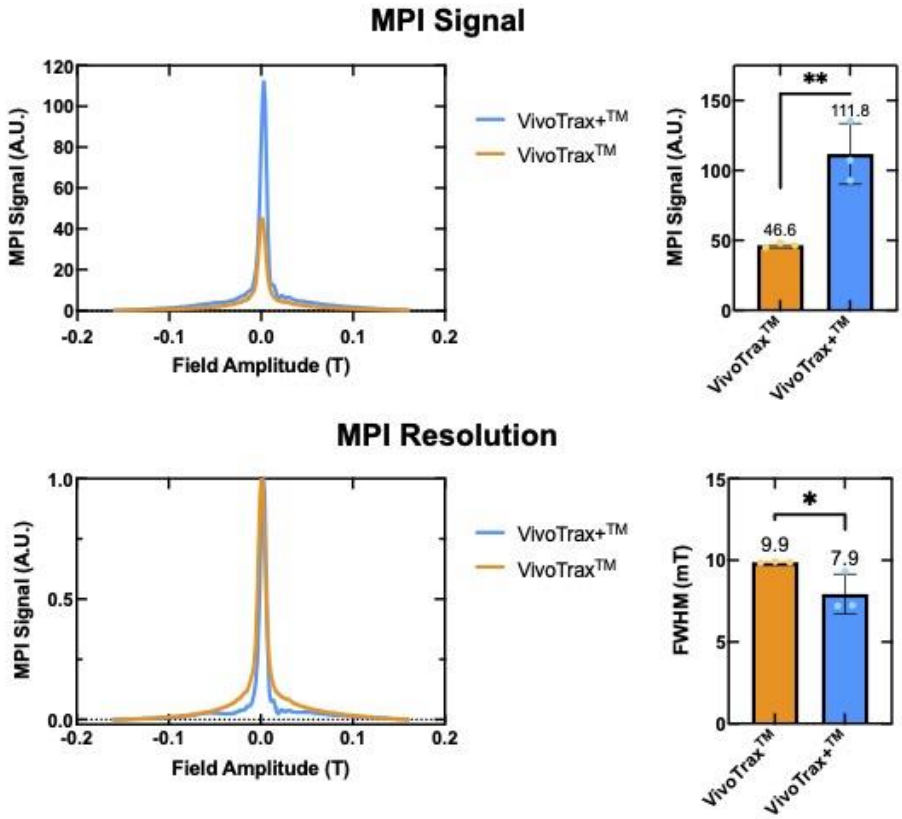

Figure 2: MPI relaxometry showing (A) signal and (B) resolution for VivoTrax+ and VivoTrax. VivoTrax+ produced significantly more MPI signal, ~2.4x, than VivoTrax (111.8 vs 46.6). Resolution is improved for VivoTrax+ by ~25% with FWHM values of 7.9 for VivoTrax+ and 9.9 for VivoTrax (* - $p \leq 0.05$, ** - $p \leq 0.01$, ordinary one-way ANOVA).

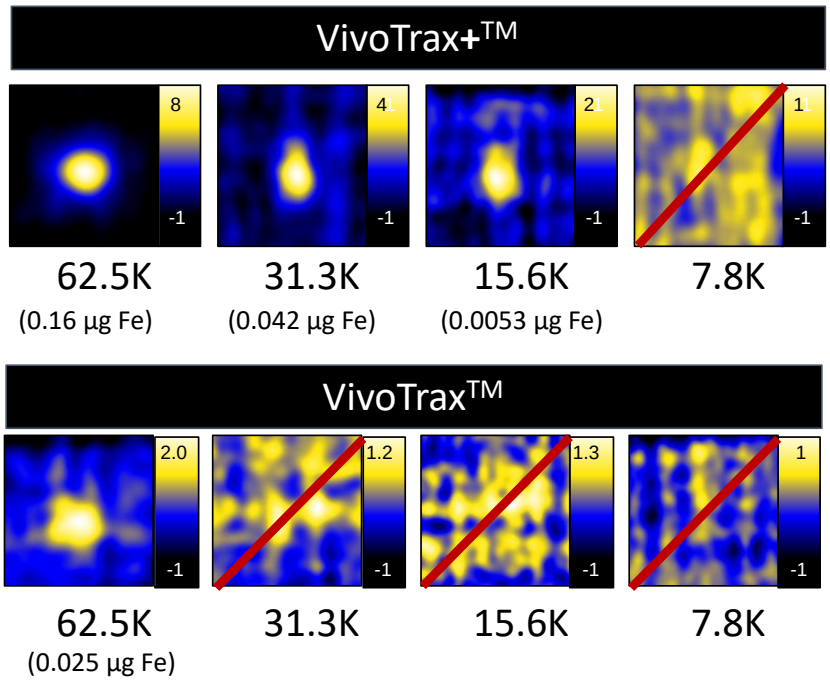

Figure 3: *In vitro* MPI of cells labeled with VivoTrax+ and VivoTrax. As few as 15.6K cells (5.3 ng Fe) were detected with VivoTrax+, ~4x fewer cells than with VivoTrax (62.5K, 25 ng Fe), using a threshold 5x that of the standard deviation of background signal. Signal below this threshold (indicated by the red line) was not measured.

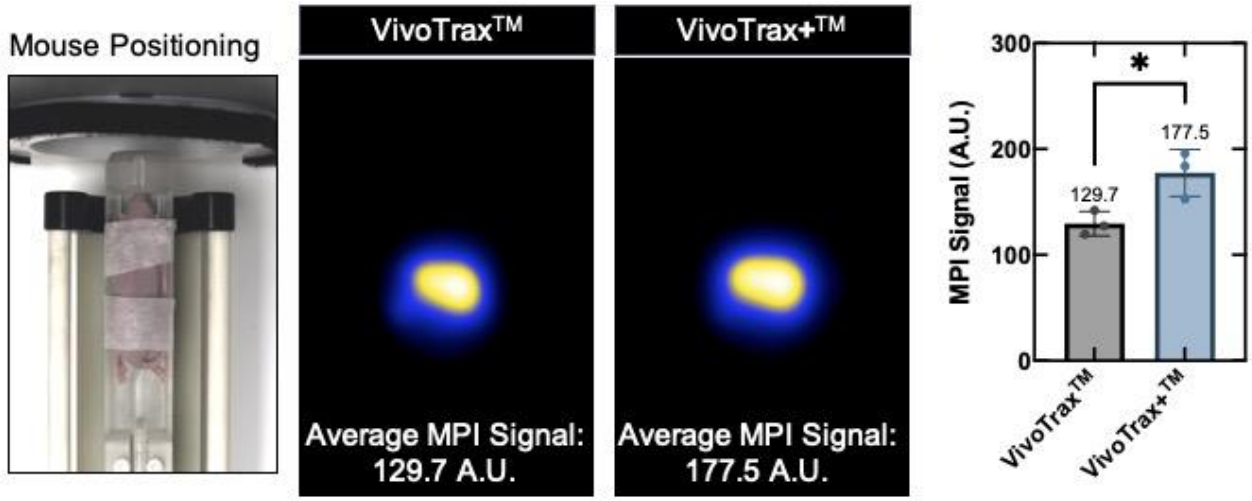

Figure 4: *In vivo* MPI signal from the mouse liver after IV injections of VivoTrax+ and VivoTrax (n = 3 per agent). The MPI signal detected in mice injected with VivoTrax+ was significantly higher than in mice injected with VivoTrax with an average MPI signal of 129.7 A.U. (VivoTrax) and 177.5 A.U. (VivoTrax+) (* - $p \leq 0.05$, ordinary one-way ANOVA).