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Rapid *in situ* labelling and tracking of neutrophils and macrophages to inflammation using antibody-functionalized MPI tracers

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Abstract

White blood cell (WBC) tracking is an imaging technique for clinical diagnoses of inflammation. WBC tracking utilises In-111 scintigraphy with *ex vivo* WBC labeling, which is cumbersome (≈1 hr prep), requires hot chemistry, and kills most WBCs within 24 hours. Magnetic particle imaging (MPI) is a tracer imaging modality that detects superparamagnetic iron oxide nanoparticles (SPIOs), providing a sensitive (≈200 cell), radiation-free alternative. As such, this work shows the first *in situ* labelling and tracking of WBCs to lipopolysaccharide-induced myositis using antibody-targeted MPI (Ab-MPI). SPIOs functionalized with Anti-Ly6G or Anti-F4/80 antibodies were used to track neutrophils or macrophages respectively, yielding differential dynamics and contrast (CNR ≈ 8-13) in imaging myositis vs. an untargeted control (ferucarbotran). These results showcase Ab-MPI as a radiation-free imaging platform for *in situ* targeting of immune cell specific antigen epitopes.

I. Introduction

White blood cell (WBC) scans are a clinical imaging procedure that allows for diagnosis of inflammation and fevers of unknown origin (FUO). By radiolabelling and tracking autologous cells, radiologists are able to visualize areas of high immune activity, which can highlight pathologies such as inflammation, infection or cancer. Clinically, WBC scans are typically done with scintigraphy using either In-111 (2.8-days half life) or Tc-99m (6 hours half life). Briefly, WBCs are isolated from a blood draw, labelled by co-incubation with the selected radiotracer, and injected intravenously into the patient. Often, the scan is performed several hours later, or even the following day. Overall, this procedure is time consuming, and requires both hot chemistry and WBC handling expertise. Moreover, studies have shown notable cell damage (e.g., chromosomal damage [1]) in radiolabelled lymphocytes. In fact, this radiation damage could be a factor in the weak specificity of WBC-In-111 studies today, generating spurious signal, and precluding WBC scans for long-term immune imaging, most notably in the evaluation of immunotherapies. While it is possible to use other tracers, most tracer modalities suffer from similar radiation concerns (e.g. positron emission tomography) or are not viable for clinical applications (e.g. optical).

Magnetic particle imaging (MPI) is an emerging non-invasive tracer imaging modality [2, 3] that directly images superparamagnetic iron oxide nanoparticles (SPIOs), localizing the tracers utilising their non-linear magnetic response and a strong magnetic selection field. MPI's lack of background signal from tissues makes it ideal for high contrast imaging. Moreover, unlike other tracer modalities, MPI offers a radiation-free image with no tissue limits and negligible signal decay, making it ideal for long-term tracking studies. Indeed, MPI has shown promise in evaluating stem-cell therapies and cancer diagnosis [4, 5], with some extension into WBC tracking. However, studies so far have focused on labelling via coincubation, requiring laborious cell isolation and labelling. Moreover, SPIO coincubation with WBCs has low labelling efficiency (2 pg/cell at 24 h depending on cell type) and can result in reactive oxygen species even at moderate loading (16 pg/cell) [6].

In this study, we utilized antibody-conjugated SPIOs for *in situ* tracking of WBCs to sites of inflammation, as a proof-of-concept demonstration of antibody-targeted MPI (Ab-MPI). We validated our experiments utilising optical and histological means, showing colocalization of iron oxide, myeloperoxidase, and neutrophil activity.

II. Methods and materials

Anti-Ly6G-antibody-conjugated SPIOs and Anti-F4/80-antibody-conjugated SPIOs (IgG1, REA526 clone, Miltenyi Biotec, GmBH) (jointly Ab-SPIOs), as well as ferucarbotran (VivoTrax™, Magnetic Insight) were processed for *in vivo* usage, and characterized with DC magnetometry, electron microscopy, and relaxometry.

For the tracking experiments, myositis was induced in seven wild-type C57BL6 mice (7-8 wks) by an intramuscular injecting 50 mg of lipopolysaccharide in the right thigh. After 24 h, three of the mice were injected intravenously with Anti-Ly6G SPIOs, one with Anti-F4/80 SPIOs, and three with ferucarbotran (all 5 mg Fe/kg, 40 µg protein/mouse). The three types of SPIOs were also injected in three control wild-type C57BL6 mice (7-8 wks).

The biodistribution at 24 h post IV injection was imaged with a 6.3 T m^{-1} field-free line MPI scanner (projection FOV 10.1 cm × 4.7 cm, t = 95 s/projection). The contralateral side of each mouse was used as healthy control. Contrast was assessed by selecting symmetric regions of interests around the central axis, and comparing the mean signal in each ROI. Myositis was validated *in vivo* with optical scans (IVIS Lumina, 5 min) after IP injections of luminol (XenoLight RediJect Inflammation Probe, Perkin Elmer) [7]. Finally, neutrophil tracking was validated with histology staining against iron and myeloperoxidase (MPO).

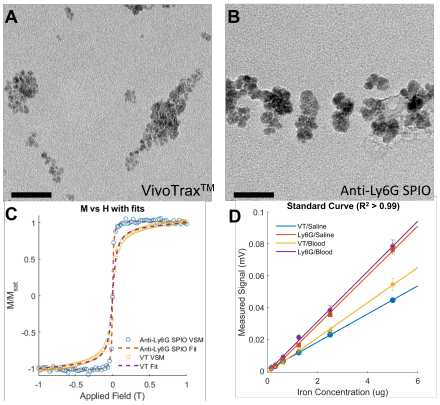

Figure 1: Physical and magnetic characterization of Ab-SPIOs and ferucarbotran. (A,B) electron microscopy images of the SPIOs. (C) DC magnetometry of the SPIOs. (D) standard curve showing SPIO behaviour in saline and blood

Tracer	D_{phys} (nm)	Resolution (mm)	D_{mag} (nm)	$\sigma_{\ln D_{mag}}$
Ab-SPIO	14 ± 2	1.26	14.8	0.5
VivoTrax™	5 ± 1	1.49	9.8	0.2

Table 1: Physical and magnetic characteristics of tracers. Resolution is given for a 7 T m^{-1} gradient, and magnetic diameter D_{mag} was fit for a lognormal particle distribution.

III. Experiments

Anti-Ly6G results were shown in [8]. SPIO characterization of Ab-SPIOs versus ferucarbotran is shown in Fig. 1, with the magnetic response from DC magnetometry and the standard curve in saline and blood (Table 1).

The tracking results are shown in Fig. 2- 4. 24 h post IV injection of SPIOs, 3D MPI-CT images showed Ly6G tracer and F4/80 tracer distribution in organs of the reticuloendothelial system (RES, e.g. liver, spleen, marrow) in healthy mice (Fig. 2). ROI analysis of MPI images of the inflamed mice with Ly6G tracer showed accumulation at the inflamed site (Fig. 3) with high contrast (CNR = 8-13 at 24 h) compared to the contralateral, uninflamed flank.

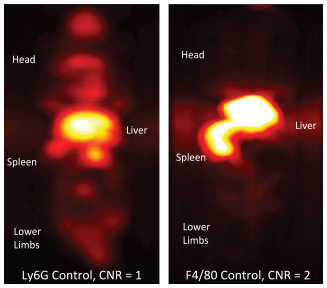

Figure 2: Healthy scans for both Ab-SPIOs. Note the accumulation in the RES organs, similar to human WBC scans [9]

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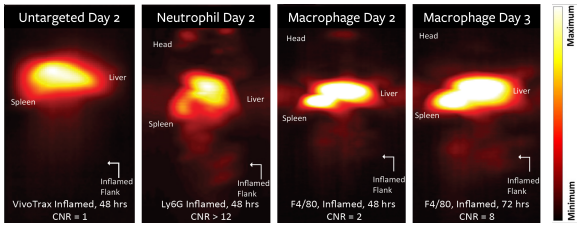

Figure 3: Inflamed MPI scans. Note the accumulation of SPIOs with neutrophils targeting on day 2, and macrophage targeting on day 3, as compared to the untargeted control.

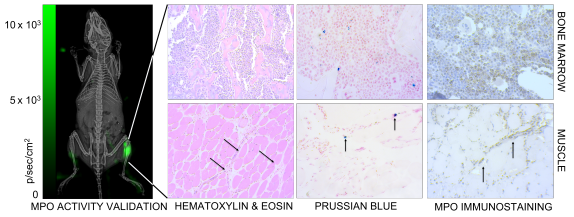

Figure 4: Validation with bioluminescence and histology. IVIS scans (left) show myeloperoxidase (MPO) activity in the inflamed flank. Histology (right) shows co-localization of iron (Prussian Blue) and MPO, indicating tracking to inflammation.

Similar accumulation and contrast (CNR = 8) was observed in the inflamed mouse with F4/80 tracer at 48 h post IV injection (Fig 3). In comparison, the inflamed mice with ferucarbotran had no contrast (Fig 3, CNR = 1). The myositis was confirmed visually in all mice (blackening of thigh), and in select mice with bioluminescence scans, indicating MPO activity. Histology from the inflamed flank showed colocalization of MPO and iron in the muscle and bone marrow (Fig. 4).

IV. Discussion

The *in vitro* and *in vivo* results highlight Ab-MPI's potential. From Fig. 1 and Table 1, the Ab-SPIOs show improved sensitivity and resolution vs. ferucarbotran. This persists in blood, making Ab-SPIOs ideal for *in vivo* use. In the *in vivo* tracking experiments, healthy MPI scans showed RES uptake, mirroring the biodistribution of In111-WBC scans [9]. In the inflamed mice, the Ab-SPIOs tracking to inflammation compared to the ferucarbotran control indicate that the contrast is unlikely due to inflammatory extravasation of SPIOs, but rather by Ab-SPIO targeting. Of note is the contrast from the anti-F4/80-SPIOs (i.e. macrophages) - contrast was patent at 72 h after inflammation. In comparison, the anti-Ly6G MPI scans (i.e. neutrophils) showed contrast at 48 h post inflammation. This is consistent with literature, and lends credence to our results [7] - neutrophils are the first responders in inflammation, and appear in the acute response (2-4 d after immune challenge). In comparison, macrophages are active in chronic inflammation (3-10 d after immune challenge) [7].

V. Conclusion

The *in vivo* Ab-MPI leukocyte tracking in this work provides a novel platform for MPI studies. The targeted tracking to sites of immune activity shows promise towards infection, immunotherapies, and bone marrow imaging. As a radiation-free modality, MPI provides a great alternative to nuclear medicine, and allows for extension to long term studies.

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Author's statement

Conflict of interest: S. M. C. is a co-founder and holds stock in an MPI company, Magnetic Insight.The authors declare no other conflict of interest. Part of this work was reproduced with permission [8].

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