Development of Iron Oxide Nanoparticles for In Situ T-Cell Labeling and Imaging

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Background

Current methods for studying T-cell trafficking often rely on ex vivo manipulation, which can alter their natural physiology. There is an unmet need for technologies that enable in vivo tracking of these cells to improve understanding of immune dynamics in disease, therapy, and transplantation.

Here, we use commercially available **anti CD4-conjugated superparamagnetic iron oxide nanoparticles (CD4-SPIONs)** to enable direct, in situ labeling of $CD4^+$ T cells via intravenous delivery.

Helper T Cells shape immune responses and can be monitored with MRI

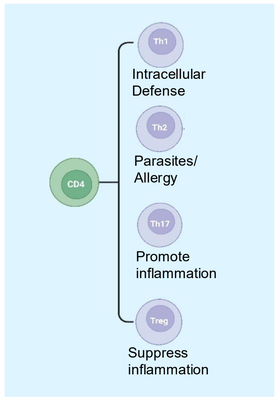


Figure 1.1: The role of CD4 T cells in the body.

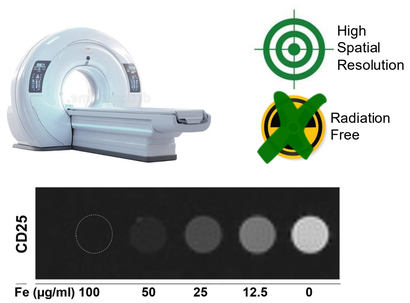


Figure 1.2: MRI is a safe imaging method, and when combined with contrast agents such as SPION there is strong soft tissue contrast.

Receptor-Targeted Uptake of SPION

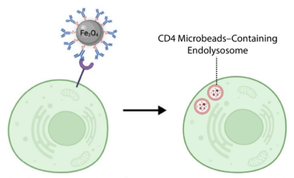


Figure 2.1: Schematic of receptor-mediated labeling

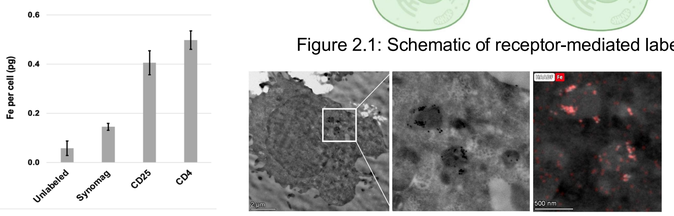


Figure 2.2: ICP-OES quantification of iron uptake per cell and TEM imaging of iron localization in endo/lysosomes, with iron-specific filter (red) on STEM mode.

Uptake by Targeted Cells, but not by Macrophages!

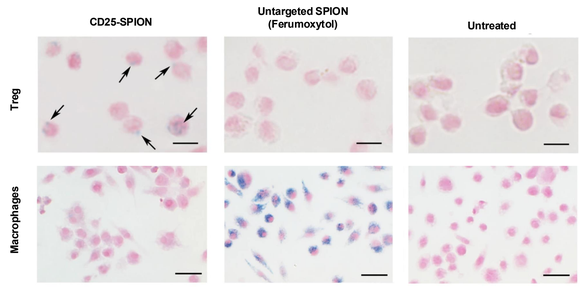


Figure 3: Treg and Macrophage cells were labeled overnight in vitro with CD25-SPION or Ferumoxytol followed by Prussian Blue iron staining.

Final Experimental Design

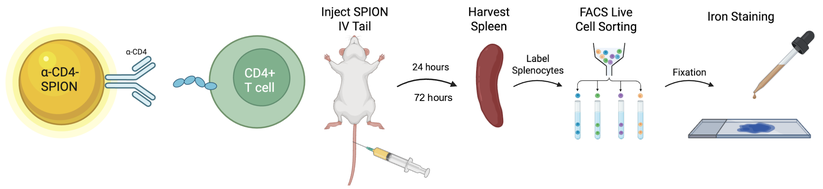


Figure 4: Schematic depicting our final design of intravenous injection of CD4-SPION followed by cell sorting and staining.

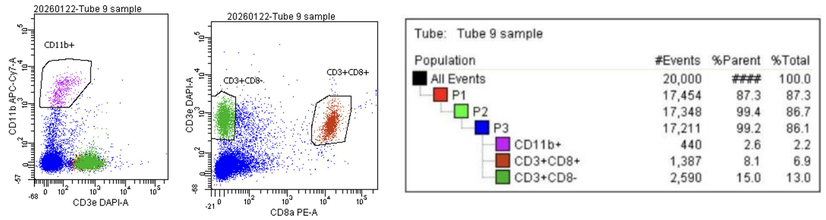
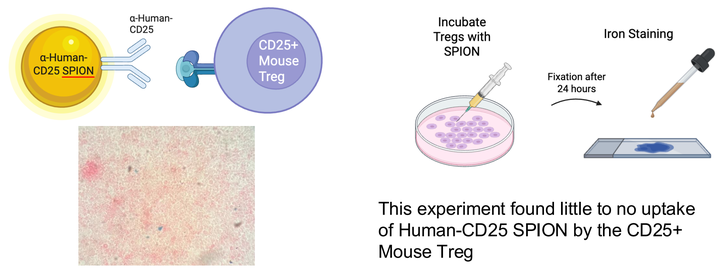


Figure 5: Representative FACS cell sorting gating strategy and concentrations of each cell subtype population.

Experimental Iterations

Targeting CD25 Mouse Treg with CD25 Human SPION



This experiment found little to no uptake of Human-CD25 SPION by the CD25+ Mouse Treg

Figure 6: Schematic of human CD25 SPION targeting mouse CD25 and images of the cells after Prussian blue iron staining.

Targeting CD25 Mouse Treg with CD25 PE-SPION

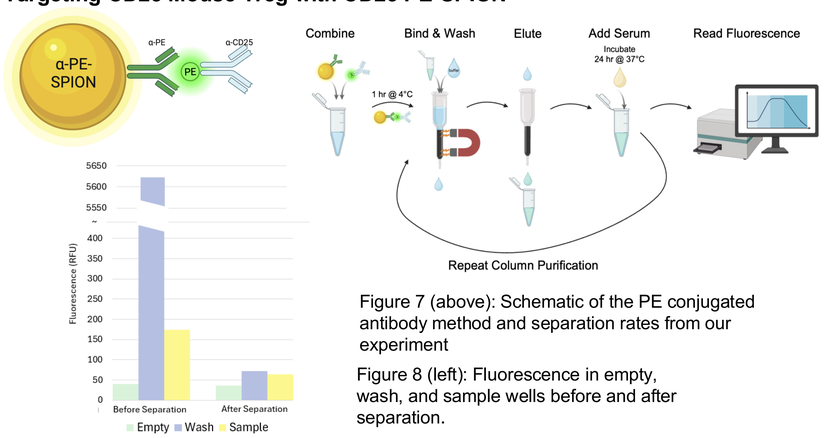


Figure 7 (above): Schematic of the PE conjugated antibody method and separation rates from our experiment

Figure 8 (left): Fluorescence in empty, wash, and sample wells before and after separation.

Outcomes

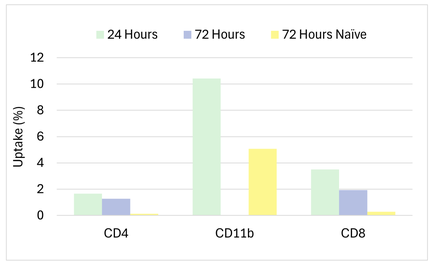


Figure 9: SPION Uptake percentage in CD4+, CD11b+, and CD8+ cells as measured by average number of cells with Prussian Blue out of total cells in image, 24 or 72 hours after CD4-SPION or PBS injection.

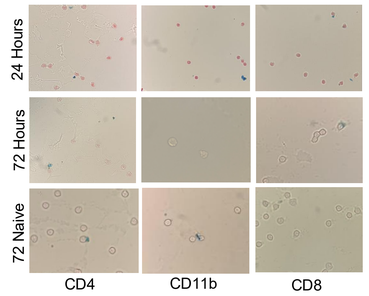


Figure 10: Representative images of the Prussian Blue stained slides under 63x magnification.

Discussion

1. Alternate SPION Test 1: Human CD25 antibody against mouse Tregs.
   - The incompatibility was likely caused by inherent epitope differences between the two species, preventing effective antibody binding.
2. Alternate SPION Test 2: Conjugated the CD25 antibody to a PE conjugated SPION
   - Fluorescence shown after separation indicates extremely low CD25 antibody retention likely due to low fluorescence binding strength.

Experiment Results: Several experiments with varied incubation times and conditions

- Observed uptake can change between 24 and 72 hours due to factors such as cell death and proliferation.
- Some observed staining could be due to contamination and potential artifacts or counting endogenous iron staining.
- Reduced cell counts in the 72 hours incubation group may have limited the statistical reliability and representativeness of the results.

Conclusions

In our experiments, we attempted to label CD4 T cells in vivo using SPIONs with limited success. The overall uptake percentage and targeting specificity remained below the desired levels for reliable clinical or research applications. Despite these limitations, the results suggest that in vivo T-cell labeling with SPIONs remains a promising approach and further nanoparticle optimization could significantly improve labeling efficiency and specificity.

Future work should investigate SPION uptake across a wider range of conditions to better understand the factors influencing nanoparticle distribution and internalization. Additional studies evaluating the long-term readability, stability, and efficacy of SPION-labeled T cells as MRI contrast agents would also be valuable. Effects on the T-cell's function must also be researched as if compromised, it would undermine the entire purpose.

Acknowledgements & References

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References: