A Novel Dual MRI-Fluorescent Contrast Agent to Track T-Cells for In-Vivo Imaging

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Introduction

Magnetic resonance imaging (MRI) provides a means to track immune cells (e.g., T-cells, macrophages, etc) *in vivo*, non-invasively, in whole tissue, and in real time. T-cells, which play an important role in immune response, are known to exhibit low incorporation efficiency with MRI contrast agents. The goal of this study is to develop a novel nano-sized iron-oxide particle (ITRI-IOPC-NH_2) containing fluorescent dyes to monitor T-cell infiltration in an acute rejection heart model in rat.

Materials and Methods

Cationic ITRI-IOPC-NH_2 particles were synthesized from ITRI-IOPC particles, which are coated with polyethylene glycol (PEG) and contain a terminal carboxyl group on the surface (1). Fluorescein isothiocyanate (FITC) and DyLight 649-N-hydroxysuccinimide (NHS) ester were individually conjugated to ITRI-IOPC-NH_2 particles, taking advantage of the amine groups (**Scheme 1**). T-cells are incubated with ITRI-IOPC-NH_2 series particles, without the use of cell penetrating peptides or transfection agents. ITRI-IOPC-NH_2-labeled-T-cells were infused into our heterotopic working transplanted rat heart models, which allow us to monitor immune-cell infiltration and to detect cardiac dysfunction during various stages of the rejection process. These labeled T-cells are characterized by *in-vivo* MRI and *ex-vivo* magnetic resonance microscopy (MRM), followed by histological analysis.

Results and Discussion

The transverse relaxivity (r_2) value of ITRI-IOPC-NH_2 particles is 250 $s^{-1}mM^{-1}$. This nano-particle is the first reported MRI contrast agent that can label T-cells with over 90% labeling efficiency **(Fig. 1)** and achieve an intracellular iron concentration of up to 0.57 pg/cell, without the using of transfection agents, HIV-1 transactivator (TAT) peptides, or electroporation. This labeling efficiency is comparable to the using of TAT peptides to deliver USPIO (0.7 pg/cell) and protamine sulfate to deliver ferumoxides (1.5 pg/cell) (2-4). MRM images of gelatin phantoms show more hypointense spots from ITRI-IOPC-NH_2-labeled-T-cells (**Fig. 2D**) than from ITRI-IOP- or ITRI-IOPC-labeled-T-cells (**Figs. 2B-C**). Thus, the cationic surface of these particles facilitates T-cell internalization. Transmission electron microscopic (TEM) images revealed that ITRI-IOPC-NH_2 particles were in cytoplasmic vacuoles of T-cells. Labeling of T-cells with ITRI-IOPC-NH_2 does not cause any measurable physiological effects on T-cell function. Localized hypointensity can be detected at the rejecting heart by *in-vivo* MRI (**Fig. 3A-B)** and *ex-vivo* MRM (**Fig. 3C-D**). Double immunofluorescent staining shows that the cells containing the ITRI-IOPC-NH_2 particles are indeed T-cells (**Fig. 4**).

Conclusion

We have synthesized a novel dual MRI-fluorescent contrast agent, ITRI-IOPC-NH_2, which shows great r_2 relaxivity and T-cell-labeling efficiency. The ITRI-IOPC-NH_2-labeled-T-cells can be detected in rejecting allograft transplanted hearts *in vivo*.

Acknowledgements

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References

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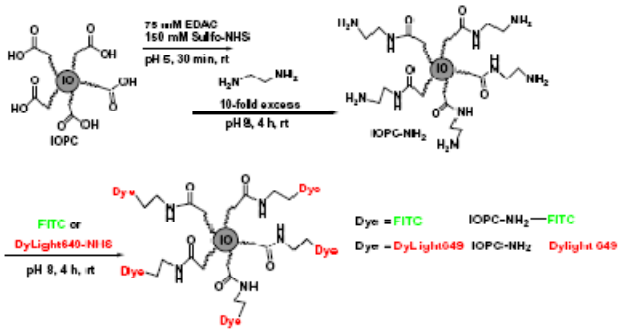

Scheme 1. Synthesis of ITRI-IOPC-NH_2, ITRI-IOPC-NH_2-FITC, and ITRI-IOPC-NH_2-DyLight649 particles.

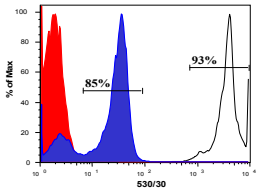

Figure 1. Flow cytometry analysis of T-cells cultured with ITRI-IOPC-NH_2-FITC particles. The peaks from left to right are: T-cells (control); T-cells after treatment with anti-CD3-FITC; T-cells after treatment with ITRI-IOPC-NH_2-FITC particles.

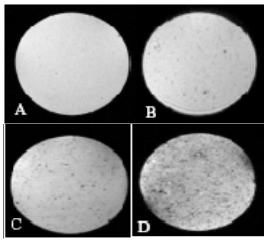

Figure 2. MRM of gelatin phantoms of 4.0x 10^6 T-cells/ml labeled with iron-oxide particles: (A) no particles (control); (B) ITRI-IOP; (C) ITRI-IOPC; (D) ITRI-IOPC-NH_2.

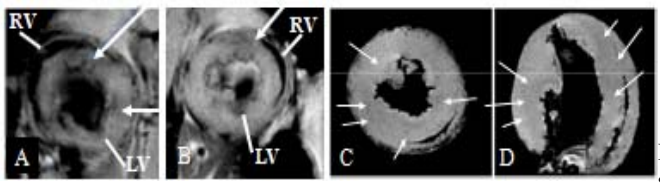

Figure 3. T_2*-weighted *in-vivo* MRI of allograft hearts on POD 4 (A) and POD 5 (B). *Ex-vivo* MRM of (C) short-axis and (D) long-axis view of allograft heart harvested on POD 6.

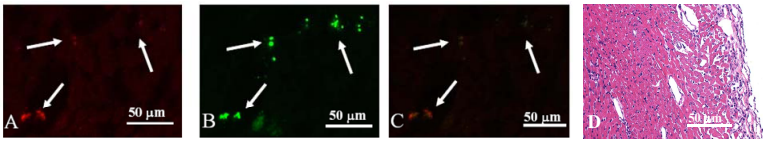

Figure 4. Fluorescent microscopic images of double immuno-fluorescent staining on frozen tissue from allograft heart. Staining with (A) PE-conjugated anti-CD3 and (B) mouse anti-PEG mAb, following treatment with anti-mouse IgG-FITC. (C) is an overlay image of A and B. (D) Optical micrograph of tissue section stained with hematoxylin/eosin for tissue integrity.