Detecting the Migration and Accumulation of Macrophages in An Acute Rejection Model of Heart-lung Transplantation in Rats by in vivo MRI Using A New Nano-sized Iron Oxide Particle

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INTRODUCTION
Iron-oxide-based cellular MRI has been used to assess organ rejection after transplantation by monitoring the accumulation of immune cells at the rejection site [1]. There are two approaches to label targeting cells with iron-oxide particles. One is in vivo labeling by direct intravenous injection, which is convenient and particularly useful for phagocytic cells, such as macrophages, which have been shown to be involved in acute transplant rejection. The other is in vitro labeling by isolating a specific cell type and labeling them in culture. Though in vitro labeling has higher sensitivity of MRI, most of the ongoing studies use focal injection after in vitro labeling interested cell types, in which the migration and accumulation of the targeting cells at the focus sites might not be convincingly demonstrated via in vivo MRI [2]. In this study, we investigated the migration and accumulation of the labeled macrophages in the transplanted allograft heart with a new, nano-sized iron-oxide-based contrast agent ITRI-IOP via in vitro labeling and venous infusion in a rat acute rejection model.

METHODS
In vitro macropage labeling by ITRI-IOP: Our nano-sized (around 60 nm) ultrasmall super-paramagnetic iron oxide particle ITRI-IOP is coated with a monolayer of polyethylene glycol (PEG). The longitudinal and transverse relaxivities of these particles are 48.9 and 321.2 s⁻¹M⁻¹, respectively. Macrophages were isolated from spleen of the recipient rat strain and labeled by ITRI-IOP in culture for 12 hrs with an iron concentration of 25 μg/ml. Perl’s Prussian Blue staining was used to confirm the presence of iron inside the cells.

Rat heart transplantation model and MRI: An abdominal working heart-lung transplantation rat model with acute rejection was used (n=9). ECG and respiration gated, single slice gradient echo imaging at the mid-ventricular wall was performed on day 5 post transplantation on a Bruker AVANCE 4.7 T system (Bruker, Billerica, MA) with the following parameters: TR = one respiration cycle (1 s), TE=8.1 ms, FOV=4×3.6 cm, in plane resolution= 156×156 µm, ST=1.2 mm, and NA=8. After a pre-scan of the transplanted allograft heart, approximately 2-30×10⁶ ITRI-IOP-labeled macrophages were infused through a catheter into the femoral vein.

RESULTS
Prior to the infusion of ITRI-IOP-labeled macrophages, the myocardial signal is uniform (Fig.1A and E). Immediately after the infusion, the transplanted lung turned into dark due to the iron-loaded macrophages in the blood circulation (Fig.1B). About 22 hrs post infusion, punctuate spots of hypointensity induced by the iron-loaded macrophages are observed on the myocardium of allograft heart and make the image look noisy (Fig.1C and G). This observation is more obvious in the ex vivo image of the same fixed transplanted heart at 4.7T (Fig.1D and H). Ex vivo images and microscopy of the transplanted and native hearts at 4.7 and 11.7 T further show abundance of punctuated spots of hypointensity only in the transplanted heart (Fig. 2 A-C, more evident at high field and high resolution), but not in the native heart (Fig. 2 D-F). The punctuate hypointensity spots in the transplanted heart are most likely due to the migration and accumulation of the ITRI-IOP-labeled macrophages from the blood circulation into the site of graft rejection. Pathology results on the corresponding tissue section also confirm the presence of co-localized iron, macrophages and rejection (Fig. 3).

CONCLUSION
In this study, we have detected the migration and accumulation of macrophages labeled in vitro by in vivo MRI with a nano-sized and sensitive iron oxide particle (ITRI-IOP) in the rejecting transplanted heart, which showed similar punctuate patterns of hypointensity as those using micron-sized particles [3]. Future studies with different immune cells specifically labeled with ITRI-IOP particles may help interpret the migration and role a specific immune cell may play in organ rejection and other inflammatory responses.