# A membrane labeling agent for MR tracking of transplanted pancreatic islets

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**Introduction:** Pancreatic islet transplant is a developing field that has shown great promise in the treatment of Type 1 diabetes, but little is known about the fate of the islets after transplant. Imaging techniques are needed to provide repeated, quantitative assessment of the viable transplanted cell mass over time. The current methods of graft assessment are indirect and may not accurately assess the effect of different treatments on the health and function of the islet cell mass; non-invasive in vivo imaging is required to study changes in islet cell mass over time. Many medical imaging methods have been considered for in vivo islet imaging, including bioluminescence and PET. However, bioluminescence is not clinically relevant due to the requirement of genetic manipulation, and limited by poor spatial resolution and penetration depth. PET has high sensitivity but poor spatial resolution, and uses radioactive tracers which are potentially harmful and have a short half-life, requiring a cyclotron near the treatment center.

MRI has emerged as a promising technique due to its high spatial resolution and non-invasive nature. Iron oxide nanoparticle agents ($T_2$ agents) have been successfully used for islet labeling and long term tracking by several groups, however the large size of the particle requires long incubation times (18-24 hr) for sufficient islet labeling. Interpretation of the signal void produced in the location of the islets can be confounded by iron deposits in the liver of some patients. Gadolinium (Gd(III)) based contrast agents ($T_1$ agents) have been understudied. An early report described a small molecule, monomeric gadolinium agent that required long incubation times in culture prior to transplant to provide sufficient signal. The goal of this work is to demonstrate the enhanced signal from a newly developed Gd-based $T_1$ MRI contrast agent that will be non-toxic to cells, label isolated islets efficiently in vitro, provide long-term labeling, and provide a strong positive signal for tracking islets post-transplant.

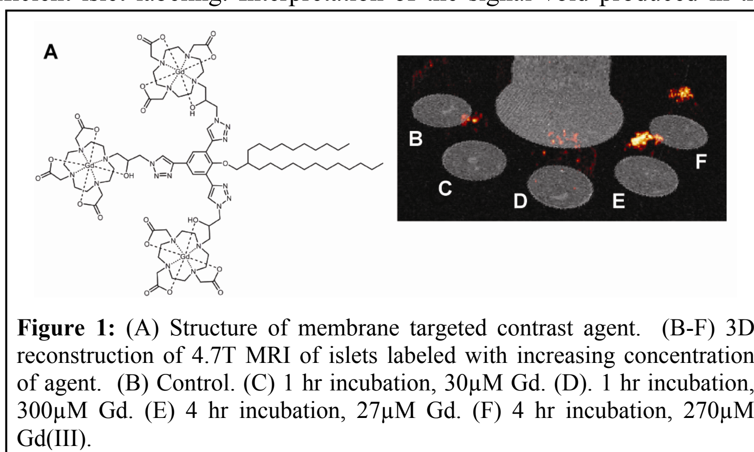

**Figure 1:** (A) Structure of membrane targeted contrast agent. (B-F) 3D reconstruction of 4.7T MRI of islets labeled with increasing concentration of agent. (B) Control. (C) 1 hr incubation, 30μM Gd. (D). 1 hr incubation, 300μM Gd. (E) 4 hr incubation, 27μM Gd. (F) 4 hr incubation, 270μM Gd(III).

**Methods:** We have designed a multimeric agent with three macrocyclic Gd(III) chelates attached to a tribromophenol-based scaffold using click chemistry, and a functional handle for further synthesis. Alkynes for click chemistry are installed on the three bromide positions, and a branched alkyne chain is installed on the phenol oxygen (**Figure 1A**).

For islet labeling studies, mouse islets were isolated and incubated in complete media (RPMI 1460 with 10% fetal bovine serum and 1% penicillin/streptomycin). Phantoms for MRI were prepared by suspending 100 islets in low melting point agarose in 1.5 mL Eppendorf tubes. MR images were acquired on a 4.7T Bruker Biospec using a 3D RARE sequence with TR/TE = 500/9.72 ms, FOV = 3.5x3.5x0.3 $cm^3$, and matrix size 256x256x64. The analysis software Amira v4.0 was used to reconstruct 3D islet images. Leaching studies were performed by incubating islets for 4h in 476 μM Gd contrast agent for 4 hours followed by an overnight incubation in fresh media. Viability studies were performed by incubating 10-50 labeled or unlabeled islets in a solution of 11 μM fluorescein diacetate (FDA) and 5 μM propidium iodide (API) in Dulbecco's Phosphate Buffered Saline. Cells were imaged on a Zeiss Axioskop fluorescent microscope.

**Results and Discussion:** The probe has three Gd(III) moieties with an $r_1$ relaxivity of 15.5 $mM^{-1}s^{-1}$ per Gd(III) and a total $r_1$ of 46.5 $mM^{-1}s^{-1}$ per molecule. The monomeric contrast agents have a relaxivity of 3.4 $mM^{-1}s^{-1}$ per Gd(III); the observed boost in relaxivity is likely due to increased $\tau_R$ (rotational correlation time) because of increased molecular weight.

As part of our pancreatic islet fate mapping research, we functionalized this agent to label islet cell membranes to both increase labeling efficiency and enhance contrast agent sensitivity (**Figure 1A**). We observed labeling in a concentration- and time-dependent fashion. Preliminary results suggest that islets can be labeled with 100-fold lower Gd(III) concentration and shorter incubation time than was necessary for agents that have been previously tested (**Figure 1B-F**).

Labeled islets could be detected by MRI at incubation concentrations as low as 30 μM Gd(III) (corresponding to ~10 μM agent). We observed no leaching of the agent from islets labeled with a high concentration of agent (476 μM Gd(III)) when placed in fresh media overnight (3.9±1.3 pmol Gd(III)/islet for leached islets versus 4.0±1.1 pmol Gd(III)/islet for unleached islets). This is an important feature that has been *lacking* in other Gd(III) based cellular labeling contrast agents. No significant reduction in cell viability was observed after labeling islets (**Figure 2**), and labeled islets appeared morphologically normal. In vivo studies of transplanted labeled islets with this agent are underway.

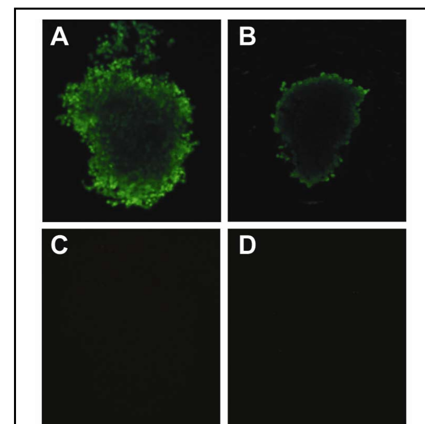

**Figure 2:** Islets remain viable after incubation with contrast agent. FDA is taken up in both control (A) and labeled (B) islets; API is excluded in both control (C) and labeled (D) islets