# Cellular Imaging of Individual Pancreatic Islets Using Electroporation and 3DFIESTA at 1.5 Tesla

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## Introduction

Type I diabetes affects ~10 million people worldwide and this number is expected to increases to 25 million by 2010. The only way to achieve permanent normoglycemia in these patients is by the renewal of insulin producing βcells through transplantation of either a vascularized pancreatic graft or isolated islets of Langerhans. In clinical practice isolated islets are transplanted by infusion into the portal vein, which exposes them to the host blood. This initiates an inflammatory reaction that ultimately causes islet damage and death. In addition this reaction stimulates a severe rejection response that requires an aggressive immunosuppressive drug protocol to control. Strategies to inhibit these cascade reactions are of great importance in improving outcome of clinical islet transplantation. To accomplish this the fate of cell transplants and the spatial, temporal and morphological features of the cellular inflammatory reaction must be better defined. Currently, there is no effective method of assessing islet transplant survival in the clinical setting. Experimentally this assessment is performed by removal of the graft and extensive histological analysis. To better understand the fate of transplanted islets an accurate and reproducible method of imaging islets *in vivo* is needed.

MRI of superparamagnetic iron oxide (SPIO) tagged cells has become a useful tool for studying cell trafficking in animal models of disease. In this study we demonstrate that individual islets can be labeled using electroporation with a clinically available SPIO and that the steady state free precession (SSFP) imaging sequence FIESTA, used in conjunction with custom-built microimaging hardware can be used to detect individual islets at 1.5T. This work provides the first evidence that tracking the fate of islets may be possible using clinical MRI scanners.

## Methods

***Islet Isolation and Labeling:*** Porcine islets of Langerhans were isolated from 7-14 days old pigs by collagenase digestion of pancreatic tissue. A novel method for islet labeling was developed which involves the use of electroporation, along with a protocol for cell labeling with polycationic transfection agents, first proposed by Frank *et al.*[2] Pig islet clusters were transfected by combining a mixture of Feridex (SPIO) 25 mg/ml and Poly-L-lysine (PLL) 1.5 mg/ml using electroporation (125-250 volts, 25 microF). After SPIO labeling, various sizes of islet cell clusters were hand-picked and suspended one by one in 2% gelatin and localized to an interface between 8% and 4% gelatin in 96-well microplates for MR and optical imaging.

***MR and Optical Imaging:*** MR imaging was performed on a 1.5T GE CV/i MR using a custom-built gradient coil (inner diameter 12cm, maximum gradient strength 600mT/m and peak slew rate 2000T/m/s) and a customized solenoidal radiofrequency coil (1cm diameter, 1cm length). Samples were scanned using 3DFIESTA (TR/TE 7.8/3.9ms, flip angle 30$^{o}$) with 100 micron isotropic spatial resolution. Scan time was 12 minutes. Optical images for each well were obtained using a stereoscope with low magnification.

***Staining and Functional Tests:*** Perl's Prussian blue staining was used to verify SPIO labeling. Immunohistochemical staining for insulin was used to investigate if SPIO affected insulin secretion. These tests were carried out on islet clusters fixed with 4% paraformaldehyde, at 18hrs after labeling. Cell viability and proliferation and the secretory response to glucose were assessed in Feridex labeled INS-1 cells, a rat beta cell line stably transfected with the human pro-insulin gene.

## Results and Discussion

**Figure 1**. Visualization of iron labeling and insulin secretion in neonatal pig pancreatic cells. Co-labeling of Perl's stain for iron (blue) and anti-insulin (DAB,brown).

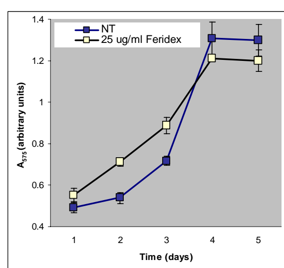



**Figure 2**. INS-1 cell proliferation after transfection with Feridex is not affected. MTT Assay; NT–nontransfected cells.

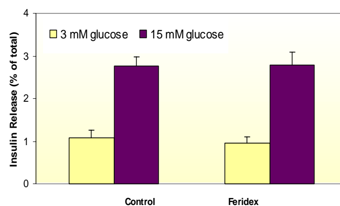
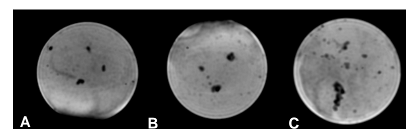



**Figure 3**. Glucose stimulated insulin secretion is preserved in Feridex transfected INS-1 cells.



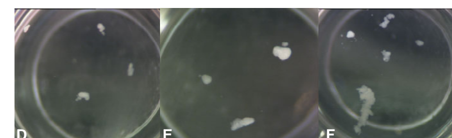




**Figure 4**. (A-C) FIESTA images of isolated, iron labeled porcine pancreatic islets suspended in gel in 50µl plastic wells. (D-F) Corresponding optical images.

The insulin secreting islet beta cell was co-stained with anti-insulin antibody for insulin expression (DAB,brown) and Prussian blue for iron, indicating that the islet cells of Langerhans were labeled with SPIO and able to secrete insulin. INS-1 cell viability, proliferation rates and insulin secretion were also not affected by Feridex labeling. Electroporation proved to be a valuable method for delivering the positively charged PLL and SPIO mixtures actively into the cell cytosol, and most importantly permitted the intracellular loading of SPIO within the islet cell mass. 3DFIESTA images acquired using our customized 1.5T microimaging system are highly sensitive to the superparamagnetic effects of iron oxide and allow the visualization of individual islets *in vitro* in short scan times. To the best of our knowledge this is the first demonstration of cellular imaging of individual pancreatic islets. *In vivo* studies are underway.

**References:** [1] Frank et al. Radiology, 228:480 (2003); [2] Foster-Gareau et al., MRM 49:968 (2003),