# Novel Radiopaque and MR-Visible Intracellular Contrast Agents for Cell-Tracking and Viability Enhancement of Human Pancreatic β-Islets

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**Introduction:** For patients with type 1 diabetes mellitus, islet transplantation offers an attractive alternative to insulin supplementation and near normal glucose control by replacing destroyed beta cells. Before transplanting islets can be employed as a suitable means for achieving insulin independence, a better understanding of the factors that affect islet graft viability and function is necessary. If transplanted islets could non-invasively be monitored using traditional imaging modalities, such as CT and MRI, these factors could be further explored. While various techniques exist to image pancreatic islets (1,2,3), imaging transplanted islets *in vivo* remains a challenge. Directly labeling cells with contrast agents offers a potential solution to overcome this issue. This abstract presents the use of directly labeling cells with MR-visible Feridex and both CT and $^{19}$F MR-visible perfluorocarbons (PFCs) to effectively track transplanted islets *in vivo*.

**Methods**: Human islets were cultured in CMRL 1066 medium supplemented with 10% fetal bovine serum, 1% penicillin/streptomycin/L-glutamine (Sigma Aldrich) in a humidified $CO_2$ incubator at 37ºC and a 5% $CO_2$ atmosphere. Islets were directly labeled with either Feridex, perfluoro-15-crown-5 ether (PFPE) or perfluorooctylbromide (PFOB). Labeled islet viability (determined using a microfluorometric assay) and function (determined by measuring insulin secretory response) were compared against unlabeled islets. PFOB labeled islets were then surgically transplanted into the kidney of mice. CT images were obtained using a Gamma Medica XSPECT scanner over a period of 30 minutes. For each scan, 1024 projections with 1024x1024 pixels were obtained at different angles of view between 0° and 360°. Scanning was performed in a clockwise direction with an X-ray tube to detector distance of 269mm and an X-ray tube to C0R distance of 225mm. Images were obtained in rotation steps of 0.703° with respective voltage and current of 50kVp and 600μA. The PFOB labeled islet recipient kidney was then excised and suspended in a gelatin phantom. MR imaging was performed on the phantom using a 9.4T MRI Scanner (Bruker BioSpin MRI GmbH), using a home-built RF probe tunable between $^{19}$F and $^{1}$H frequencies. A standard $T_2$ weighted spin echo (SE) pulse sequence was employed. Sixteen images were acquired for both anatomical proton and fluorine imaging using the SE parameters: TR/TE = 1500/15 ms; FOV 3x3 cm; matrix 128x64 pixels; slice thickness 1 mm; NA=1, total scan time 96 Seconds. Segmentation and reconstruction for both MR and CT scans were done using the imaging software Amira.

**Results**: We found that both Feridex and PFC labeled cells did not negatively affect islet viability or function using microfluorometric, insulin production, and glucose responsiveness assays when compared to unlabeled control islets. Instead, PFOB and PFPE labeled islets displayed a statistically significant increase ($p < 0.05$) in viability in comparison to their non-labeled counterparts. An MTS assay displayed a 13% increase in Feridex-labeled and a 31% increase in PFC-labeled islet proliferation when compared to unlabeled control islets. Using CT imaging, PFOB labeled islets were detectable *in vivo* after transplantation into the kidney of mice (fig. 1). While individual islets could not be resolved, small groups of islets were clearly identifiable. Under high resolution $^{19}$F MRI, PFOB labeled islets were clearly identifiable and when overlaid on anatomical $^{1}$H MRI scans, islets were easily distinguishable from soft tissue after transplantation (fig 2).

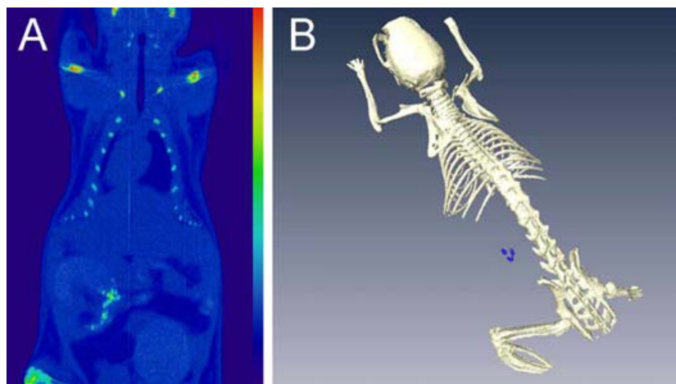


**Figure 1:** CT of mouse with PFOB labeled islets in the kidney
A) Single plane B) 3d reconstruction

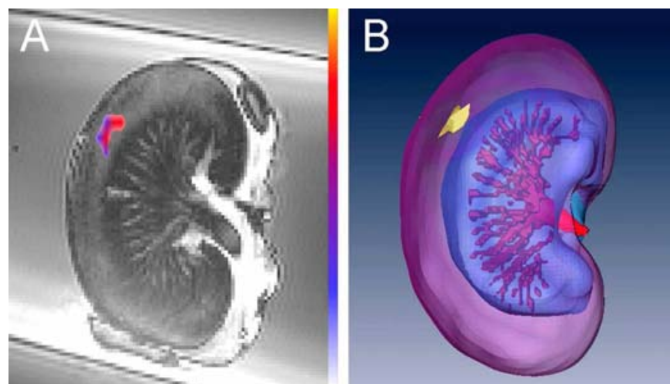


**Figure 2:** $^{19}$F MRI / $^{1}$H MRI overlay of mouse kidney with PFOB labeled islets
A) Single plane B) 3d Reconstruction

**Discussion:** By directly labeling islets with CT and $^{19}$F MRI visible PFOB emulsions, we have developed a way to effectively track cells *in vivo* after transplantation. PFOB, marketed as an oxygen carrying liquid drug, increases the viability of labeled islets making it a suitable alternative to traditional $^{1}$H MRI contrast agents such as lanthanide complexes and iron which may have unwanted toxicity effects. Moreover, the lack of endogenous fluorine ensures the specificity of the signal when imaging with $^{19}$F MRI. In addition, overlaying $^{19}$F MRI scans on anatomical $^{1}$H MRI scans taken during the same session allows for 'hot-spot imaging' (4) and accurately confirms the location of transplanted islets even when in soft tissue. The visibility of labeled cells with CT overcomes the small field of view limitations associated with MRI and also allows groups of labeled islets to be distinguishable from bone. In clinical applications, transplanted islets could be distinguished from skeletal anatomy using CT followed by confirmation using $^{19}$F MRI. As superimposition of CT and MRI scans, using hybrid X-Ray/MR imaging systems (5), become more prevalent in the future, multimodal contrast agents, such as PFOB, will allow researchers and clinicians to accurately monitor labeled cells *in vivo*.

**References:** ***(1)** Jirak, D. et al. Magn. Reson. Med. 52(6):1228-33 (2004).* ***(2)** Lu Y. et al. Proc. Natl. Acad. Sci. USA. 103(30):11294-9 (2006).* ***(3)** Evgenov, NV. et al. Nat. Med. 21(1): 144-8 (2006).* ***(4)** Bulte, J.W. Nat. Biotech. 23, 945-946 (2005).* ***(5)** Ganguly, A. et al. Acad. Radiol. 2005. 12(9):1167-77.*

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