

Clinically Relevant Imaging of Transplanted Pancreatic Islet Cells

Dian R. Arifin

Department of Chemistry, Towson University, Baltimore, Maryland, USA

Received: 01 May, 2017; Accepted: 24 May, 2017; Published: 02 June, 2017

*Corresponding author: Dian R. Arifin, Department of Chemistry, Towson University, Baltimore, Maryland, USA; E-mail: dianrarifin@yahoo.com

Abstract

Transplantation of pancreatic islet cells has shown exciting potential for normalizing the blood glucose levels of diabetic patients and those suffering from chronic pancreatitis. The underlying causes of transplant failure and variations in success rate however are poorly understood. At present, islets are transplanted via minimally-invasive infusion into a “black box,” that is the fate of islets after infusion into patients is not known. A promising approach uses medical imaging technology to monitor, study and evaluate islet grafts during transplantation and *in situ* in a clinically relevant and non-invasive fashion, hence offering a means to apply timely medical intervention in the case of therapy failure and to improve the long-term outcome of islet transplantation. This review focuses on advancements in clinical imaging of transplanted islets as well as the limitations and advantages of each imaging modality.

Introduction

Transplantation of pancreatic islet cells has shown exciting potential for normalizing the blood glucose levels of type I and severe type II diabetic patients and those suffering from chronic pancreatitis. Unlike daily insulin injections, transplanted islets can respond to elevation of systemic blood glucose levels in a proportional and rapid manner, therefore removing any adverse effects due to inaccurate doses of insulin. Although human islet isolation and subsequent transplantation following the Edmonton protocol had been undergoing multiple trials in the clinics, the percentages of recipients achieving insulin independence, the period of insulin independence and the health complications accompanying islet transplantation significantly varied between clinics and trials [1-6]. The underlying causes of therapy failure and variations in success rate are poorly understood.

At present, islets are transplanted via minimally-invasive infusion into a “black box,” that is the fate of islets after infusion into patients is not known. Conventional methods of analyzing insulin, c-peptide and glucose levels in the blood samples are inadequate since the results are influenced by physiological changes and these methods can only detect islet death or impaired function only after the events. A promising approach uses medical imaging technology to monitor, study and evaluate islet grafts during transplantation and *in situ* in a clinically relevant and non-invasive fashion. Moreover, ability to

track islets ensures that islets are delivered to the correct target areas. Such approach may also enable temporal and spatial study of islet grafts, hence offering a means to apply timely medical intervention in the case of therapy failure and to improve the long-term outcome of islet transplantation. This review focuses on advancements in clinical imaging of transplanted islets as well as the limitations and advantages of each imaging modality.

Single-Photon Emission Computed Tomography (SPECT) and Positron Emission Tomography (PET)

SPECT and PET are nuclear medicine imaging modalities commonly used to study metabolic processes in the body. Both scanners detect radioactive materials as “hot spots”, providing high sensitivity and good resolution with almost no background interference. A SPECT or PET scan is typically combined with anatomical images from MRI or CT/X-ray imaging. SPECT directly detects gamma radiation emitted by radioisotopes, whereas PET detects pairs of gamma rays emitted indirectly by a positron-emitting radiotracers [7]. PET offers higher sensitivity and better spatial resolution than SPECT. However, SPECT radiotracers and gamma scanning equipment are less expensive than PET. SPECT radiotracers have longer decay time than those of PET, thus allowing a longer observation window. In addition, PET is less accessible than SPECT since a cyclotron is required to produce PET radiotracers and an immediate transport from the production site to the imaging site is necessary due to the rapid decay time of PET radioisotopes. For islet imaging, SPECT or PET radiotracers are designed to target and label islets *in vivo* with high specificity. SPECT radiotracer [123I]-iodobenzamide (IBZM) and [111In]-exendin-3 target dopamine 2 receptor and glucagon like peptide-1 receptor, respectively, which are expressed on islet cells. Islets engrafted in rats could be serially imaged by SPECT for 4-10 weeks after infusion of [123I]-IBZM or [111In]-exendin-3. Imaging of individual islets was a possibility due to its high detection sensitivity [8-10]. In addition, SPECT may facilitate quantification of volume or mass of islet grafts as SPECT signal correlated linearly with islet volume or mass. This method therefore can potentially be used for *in situ*, non-invasive and serial quantification of islet viability *in vivo* as dead islets are degraded and cleared by the recipient’s body and can be detected as signal loss.

The PET counterparts include [¹⁷⁷Lu]-DO3A-VS-Cys40-exendin-4 and [¹⁸F]-TTCO-Cys40-exendin-4 - both targeting glucagon like peptide-1 receptor- and [¹⁸F]-L-3,4-dihydroxyphenylalanine(DOPA), targeting dopaminergic metabolic pathway present in islets [11-13]. Transplanted islets in mice could be visualized by PET after infusion of said radiotracers. As PET signal correlated with the number of islet grafts, PET scan may be used for *in vivo* quantification of islets. An alternative method was to label islets with 18F-fluorodeoxyglucose (FDG) *ex vivo* prior to transplantation [14]. Although labeled islets engrafted in rats could be imaged by PET, the observation window was short (within hours) and islets had to be transplanted as soon as the labeling process was completed due to the rapid decay time of PET radioisotope.

The downside of SPECT and PET modality is the use of a radioisotope which may be toxic to the patients as well as the islets themselves. The facility to produce radiotracers is costly and is not available near small clinics and in remote areas. Moreover, the specificity of the radiotracers to exclusively label islets *in vivo* requires improvements.

Magnetic Resonance Imaging (MRI)

MRI is a medical imaging technique that can provide a 3D anatomical information of a patient's whole body with an excellent soft tissue contrast and a spatial resolution close to the size of a single cell [7]. MRI manipulation is mostly performed on water protons (1H), taking advantages of its abundance in human and animal bodies. To impart 1H MRI-visibility, islets were labeled with carbon nanotubes, commercial super paramagnetic iron oxide nanoparticles, such as Endorem, Ferucarbotran and Ferumoxytol, or gadolinium-based contrast agent *ex vivo* before transplantation [15-19]. Many studies geared toward functionalization of iron oxide nanoparticles to enhance efficiency of islet labeling are in progress [20-23]. Likewise, novel MRI protocol to improve detection of iron oxide-labeled islets is currently being developed and tested in human patients [24].

Longitudinal imaging of iron oxide-labeled islets by ¹H MRI as dark (hypointense) entities had been successfully demonstrated in murine models up to 2-8 weeks, in nonhuman primate models as well as in patients up to 10 months post-transplantation [15,17,22, 25, 18]. MRI of islets labeled with gadolinium-based contrast agents showed visibility as bright (hyperintense) signals *in vitro* although *in vivo* detection was inconclusive [19]. Iron oxide and gadolinium-based contrast agents do not degrade over time, thus offering long shelf-lives and stable signal intensity as long as the contrast agents are retained by islets [7].

MRI for quantification of islet grafts and predicting therapy success or failure remains a challenge [26]. Recent findings however are promising. In murine models, a correlation between the total area of visualized islets and the transplanted islet mass was observed [16]. Disappearance of MRI signals was associated with the loss of islet graft function in nonhuman primates and in patients [25,18]. Efficient islet labeling to achieve high detection sensitivity - particularly in clinical applications - is still an issue, necessitating an improvement in labeling protocol and contrast agent. Labeled islets appeared under MRI scanner as

dark (hypointense) or bright (hyperintense) signals against black and white tissue background which may lead to misinterpretation of data. Furthermore, a MRI scanner is very expensive, making it less accessible.

Ultrasound Imaging (US)

Ultrasonography is safe, fast and the most inexpensive clinical imaging modality. The equipment is portable, therefore suitable for bed-side uses, and is available in small clinics. Recent work reported that transplanted islets could be detected as hyperechoic signals in mice by high-frequency ultrasonography (HF-US) as well as in a human patient by clinical ultrasonography [27,28]. In murine study, islet volume calculated by the HF-US device correlated with numbers of transplanted islets, while islets rejected by the host immune system were imaged as hypoechoic areas [27]. Individual islets inside a patient's portal vein could be visualized by intraoperative US with a central frequency of 7.5 MHz [28]. These data demonstrated a potential for *in vivo* monitoring, prediction of transplant rejection and quantification of islets grafts by US. In addition, US imaging does not require islets to be labeled, hence eliminating any potential toxicity caused by contrast agents. This modality however can only image a specific area of the body, necessitating prior knowledge of the location of transplanted islets. Islets grafts appeared as dark entities, and thus may be difficult to be differentiated from the black and white tissue background.

A Hybrid of Imaging and Encapsulation Method

A multifunctional approach is encapsulation of islets inside labeled, semi-permeable, spherical biomatrix. The biomatrix provides a 3D supportive environment for the islets while acting as a physical barrier to protect transplanted islets against invasion of the immune system. Islet encapsulation allows indirect labeling of transplants by labeling the biomatrix instead of the islets which may reduce the contrast agent's potential cytotoxicity to the islets themselves. The volume of the biomatrix allows higher concentration of contrast agents to be used which in turn improves the detection sensitivity of the transplants. MRI, X-ray and US-compatible encapsulated islets have been developed up to date. Encapsulated islets labeled with iron oxide nanoparticles and engrafted into various transplantation sites in swine models were visible under a clinical 1.5T MRI scanner [29]. Microcapsules synthesized using barium or labeled with gold nanoparticles could be imaged by CT/X-ray scanner or X-ray phase contrast imaging in rodents [30-32]. Those labeled with gadolinium-gold nanoparticles and engrafted into murine models can be imaged in a trimodal fashion - simultaneously by MRI, CT/X-ray and ultrasonography - and thus combining the advantages of each imaging modality [33]. Due to its high detection sensitivity, this hybrid approach offers the potential of individual capsule imaging *in vivo*, i.e. individual islet imaging since each capsule enclosed approximately one islet. However, immune rejection caused by the biomatrix itself is an issue and efforts to develop a biocompatible material for encapsulation are in progress [29].

Despite advancements in islet labeling protocol and imaging technology, clinical imaging of transplanted islets remains

an experimental method which requires further research in contrast agent toxicity, stability of contrast agents or imaging signals over the observation period, detection sensitivity and reliable methods for *in vivo* quantification of islet grafts.

References

- Shapiro AMJ, Lakey JRT, Ryan EA, Korbutt GS, Toth E, Warnock GL, et al. Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. *N Engl J Med*. 2000;343(4):230-238. doi: 10.1056/NEJM200007273430401
- Peters A, Olateju T, Deschenes J, Shankarnarayan SH, Chua N, Shapiro AM, et al. Post-Transplant Lymphoproliferative Disorder Following Clinical Islet Transplantation: Report of the First Two Cases. *Am J Transplant*. 2017. doi: 10.1111/ajt.14303
- Lara LF, Takita M, Burdick JS, DeMarco DC, Pimentel RR, Erim T, et al. A study of the clinical utility of a 20-minute secretin stimulated endoscopic pancreas function test and performance according to clinical variables. *Gastrointest Endosc*. pii: S0016-5107(17)31768-6. doi: 10.1016/j.gie.2017.03.1532
- Jackson S, Mager DR, Bhargava R, Ackerman T, Imes S, Hubert G, et al. Long-term follow-up of hepatic ultrasound findings in subjects with magnetic resonance imaging defined hepatic steatosis following clinical islet transplantation: a case-control study. *Islets*. 2013;5(1):16-21. doi: 10.4161/isl.24058
- Johnston PC, Lin YK, Walsh RM, Bottino R, Stevens TK, Trucco M, et al. Factors associated with islet yield and insulin independence after total pancreatectomy and islet cell autotransplantation in patients with chronic pancreatitis utilizing off-site islet isolation: Cleveland Clinic experience. *J Clin Endocrinol Metab*. 2015;100(5):1765-1770. doi: 10.1210/jc.2014-4298
- Maffi P, Berney T, Nano R, Niclauss N, Bosco D, Melzi R, et al. Calcineurin inhibitor-free immunosuppressive regimen in type 1 diabetes patients receiving islet transplantation: single-group phase 1/2 trial. *Transplantation*. 2014;98(12):1301-1309. doi: 10.1097/TP.0000000000000396
- Arifin DR, Bulte JWM. Nanobiomaterials for molecular imaging. In *Nanobiomaterial Handbook*. Sitharaman B. (Ed.). CRC Press, Taylor & Francis Group. 2011;17.1-17.16.
- Willekens SM, van der Kroon I, Bos D, Joosten L, Frielink C, Boerman OC, et al. Quantitative and longitudinal imaging of intramuscular transplanted islets of Langerhans with SPECT using [123 I]IBZM. *Diabetes Obes Metab*. 2017;19(4):604-608. doi: 10.1111/dom.12857
- Willekens SM, van der Kroon I, Joosten L, Frielink C, Boerman OC, van den Broek SA, et al. SPECT of Transplanted Islets of Langerhans by Dopamine 2 Receptor Targeting in a Rat Model. *Mol Pharm*. 2016;13(1):85-91. doi: 10.1021/acs.molpharmaceut.5b00518
- van der Kroon I, Andralojc K, Willekens SM, Bos D, Joosten L, Boerman OC. Noninvasive Imaging of Islet Transplants with 111In-Exendin-3 SPECT/CT. *J Nucl Med*. 2016;57(5):799-804. doi: 10.2967/jnumed.115.166330
- Espes D, Selvaraju R, Velikyan I, Krajcovic M, Carlsson PO, Eriksson O. Quantification of β -Cell Mass in Intramuscular Islet Grafts Using Radiolabeled Exendin-4. *Transplant Direct*. 2016;2(8):e93. doi: 10.1097/TXD.0000000000000598
- Wu Z, Liu S, Hassink M, Nair I, Park R, Li L, et al. Development and evaluation of 18F-TTCO-Cys40-Exendin-4: a PET probe for imaging transplanted islets. *J Nucl Med*. 2013;54(2):244-251. doi: 10.2967/jnumed.112.109694
- Eriksson O, Mintz A, Liu C, Yu M, Naji A, Alavi A. On the use of [18F] DOPA as an imaging biomarker for transplanted islet mass. *Ann Nucl Med*. 2014;28(1):47-52. doi: 10.1007/s12149-013-0779-4
- Otsuki K, Ito T, Kenmochi T, Maruyama M, Akutsu N, Saigo K, et al. Positron Emission Tomography and Autoradiography of (18)F-Fluorodeoxyglucose Labeled Islets With or Without Warm Ischemic Stress in Portal Transplanted Rats. *Transplant Proc*. 2016;48(1):229-233. doi: 10.1016/j.transproceed.2015.12.025
- Syed F, Riggio C, Masini M, Bugliani M, Battaglia V, Novelli M, et al. Labeling and Tracking of Human Pancreatic Islets Using Carbon Nanotubes. *J Biomed Nanotechnol*. 2015;11(4):730-738.
- Jin SM, Oh SH, Oh BJ, Shim W, Choi JM, Yoo D, et al. Feasibility of islet magnetic resonance imaging using ferumoxytol in intraportal islet transplantation. *Biomaterials*. 2015;52:272-280. doi: 10.1016/j.biomaterials.2015.02.055
- Jin SM, Oh SH, Oh BJ, Suh S, Bae JC, Lee JH, et al. Benefits of PEGylation in the early post-transplant period of intraportal islet transplantation as assessed by magnetic resonance imaging of labeled islets. *Islets*. 2014;6(1):e27827. doi: 10.4161/isl.27827
- Malosio ML, Esposito A, Brigatti C, Palmisano A, Piemonti L, Nano R, et al. MR imaging monitoring of iron-labeled pancreatic islets in a small series of patients: islet fate in successful, unsuccessful, and auto transplantation. *Cell Transplant*. 2015;24(11):2285-2296. doi: 10.3727/096368914X684060
- Berkova Z, Jirak D, Zacharovova K, Lukes I, Kotkova Z, Kotek J, et al. Gadolinium- and manganite-based contrast agents with fluorescent probes for both magnetic resonance and fluorescence imaging of pancreatic islets: a comparative study. *ChemMedChem*. 2013;8(4):614-621. doi: 10.1002/cmdc.201200439
- Kitamura N, Nakai R, Kohda H, Furuta-Okamoto K, Iwata H. Labeling of islet cells with iron oxide nanoparticles through DNA hybridization for highly sensitive detection by MRI. *Bioorg Med Chem*. 2013;21(22):7175-7181. doi: 10.1016/j.bmc.2013.08.063
- Wang Y, Blanco-Andujar C, Zhi ZL, So PW, Thanh NT, Pickup JC. Multilayered nanocoatings incorporating super paramagnetic nanoparticles for tracking of pancreatic islet transplants with magnetic resonance imaging. *Chem Commun (Camb)*. 2013;49(65):7255-7257. doi: 10.1039/c3cc43512a
- Juang JH, Shen CR, Wang JJ, Kuo CH, Chien YW, Kuo HY, et al. Magnetic resonance imaging of mouse islet grafts labeled with novel chitosan-coated superparamagnetic iron oxide nanoparticles. *PLoS One*. 2013;8(4):e62626. doi: 10.1371/journal.pone.0062626
- Hwang JH, Noh YW, Choi JH, Noh JR, Kim YH, Gang GT, et al. In vivo imaging of islet transplantation using PLGA nanoparticles containing iron oxide and indocyanine green. *Magn Reson Med*. 2014;71(3):1054-1063. doi: 10.1002/mrm.24768
- Deligianni X, Jirák D, Berková Z, Hájek M, Scheffler K, Bieri O. In vivo visualization of cells labeled with superparamagnetic iron oxides by a sub-millisecond gradient echo sequence. *MAGMA*. 2014;27(4):329-337. doi: 10.1007/s10334-013-0422-3

25. Wang P, Schuetz C, Vallabhajosyula P, Medarova Z, Tena A, Wei L, et al. Monitoring of Allogeneic Islet Grafts in Nonhuman Primates Using MRI. *Transplantation*. 2015;99(8):1574-1581. doi: 10.1097/TP.0000000000000682
26. Liu Y, Song B, Ran X, Jiang Q, Hu J, Vance Chiang SM. Molecular imaging of pancreatic islet transplantation. *Exp Clin Endocrinol Diabetes*. 2014;122(2):79-86. doi: 10.1055/s-0033-1363232
27. Sakata N, Kodama T, Chen R, Yoshimatsu G, Goto M, Egawa S, et al. Monitoring transplanted islets by high-frequency ultrasound. *Islets*. 2011;3(5):259-266.
28. Sakata N, Goto M, Gumpei Y, Mizuma M, Motoi F, Satomi S, Unno M. Intraoperative ultrasound examination is useful for monitoring transplanted islets: a case report. *Islets*. 2012;4(5):339-342. doi: 10.4161/isl.22384
29. Arifin DR, Valdeig S, Anders RA, Bulte JW, Weiss CR. Magnetoencapsulated human islets xenotransplanted into swine: a comparison of different transplantation sites. *Xenotransplantation*. 2016;23(3):211-221. doi: 10.1111/xen.12235
30. Arifin DR, Manek S, Call E, Arepally A, Bulte JW. Microcapsules with intrinsic barium radiopacity for immunoprotection and X-ray/CT imaging of pancreatic islet cells. *Biomaterials*. 2012;33(18):4681-4689. doi: 10.1016/j.biomaterials.2012.03.008
31. Astolfo A, Qie F, Kibleur A, Hao X, Menk RH, Arfelli F, et al. A simple way to track single gold-loaded alginate microcapsules using x-ray CT in small animal longitudinal studies. *Nanomedicine*. 2014;10(8):1821-1828. doi: 10.1016/j.nano.2014.06.008
32. Appel AA, Ibarra V, Somo SI, Larson JC, Garson AB 3rd, Guan H, et al. Imaging of Hydrogel Microsphere Structure and Foreign Body Response Based on Endogenous X-Ray Phase Contrast. *Tissue Eng Part C Methods*. 2016;22(11):1038-1048. doi: 10.1089/ten.TEC.2016.0253
33. Arifin DR, Long CM, Gilad AA, Alric C, Roux S, Tillement O, et al. Trimodal gadolinium-gold microcapsules containing pancreatic islet cells restore normoglycemia in diabetic mice and can be tracked by using US, CT, and positive-contrast MR imaging. *Radiology*. 2011;260(3):790-798. doi: 10.1148/radiol.11101608