Tri-modal in vivo imaging of the rodent pancreatic islets transplanted in the subcutaneous site

Sayuan Liang[1], Karim Louchami[1,2], Bryan Holvoet[1], Rein Verbeke[3], Bella Manshian[1], Willy J Malaisse[2], Abdullah Sener[2], Ine Lentacker[3], and Uwe Himmelreich[1]
[1]*Department of Imaging & Pathology, KU Leuven, Leuven, Flemish Brabant, Belgium,* [2]*Laboratory of Experimental Hormonology, Université Libre de Bruxelles, Brussels, Belgium,* [3]*Laboratory for General Biochemistry and Physical Pharmacy, Ghent University, Ghent, East Flanders, Belgium*

Introduction

Outcomes of beta cell islet transplantation at subcutaneous sites have been disappointing due to hypoxia-induced oxidative stress by poor vascularization. However, subcutaneous engraftment still has advantages like the simple surgical procedure under local anesthesia and the possibility to biopsy when needed[1]. Therefore, it is important to have non-invasive imaging techniques available to monitor the exact location and fate of transplanted islets. Subcutaneously transplanted islets offer the unique opportunity to perform in vivo optical imaging, which is often limited by the short penetration depth of light when using intraportal transplantations. For validation purposes, ^{19}F MRI using perfluorocarbon based particles can be used, which draws attention in the field of cellular imaging[2] thanks to the lack of background signal and possibility for quantification of transplanted cells. These perfluorocarbon particles could easily be tacked with a fluorescent dye to be also detected by fluorescence imaging. To perform BLI, islets/cells need to be transduced by viral vectors. The present work deals with the use of multimodal in vivo imaging (BLI / Fluorescence /^{19}F MRI) to image and monitor the transplanted pancreatic islets in mice. By combining different imaging techniques, we want to overcome the limitations in sensitivity, resolution and specificity of the individual method.

Materials and methods

Perfluorocarbon (PFCE) particle and lentiviral vector (LV): Cationic PFCE particles were prepared according to previous report[3] with 3.5mol% red DiD fluorescent dye (particle concentration: $4.5*10^9$ particles/ml, particle mean size: ±0.513μm). Lentiviral vector carrying the hEFlα promoter to drive triple flag tagged firefly luciferase (3flagFluc) and a puromycin resistance gene (PuroR) was used for cell/islet transduction[4]. Cell line and pancreatic islets isolation: Tumor insulin producing cells (INS-1E) were used for uptake validation as well as pancreatic islets isolated from Wistar rats (female, 8-10 weeks) using the collagenase digestion method. In-vitro studies: Phantoms containing PFCE labeled cells/pancreatic islets filled with 2% agarose were made for performing ^{19}F MRI. PFCE labeled or LV transfected cells/islets were placed in 24 well plates for either fluorescent imaging or BLI. Animal model: C57Bl6 mice (male, 8-10 weeks) were subcutaneously injection of either 200 PFCE labeled/LV transfected pancreatic islets or one million PFCE labeled/LV transfected cells at one side with another 200μl PBS injected at the other side as negative control. Microscopy and Confocal imaging: OLYMPUS CKX41 Microscope (Tokyo, Japan) was used for cell/pancreatic islets visualization. Confocal images were also acquired using a Nikon A1R Eclipse Ti confocal microscope (Tokyo, Japan). NMR spectroscopy: NMR experiments were carried out using a 400 MHz Bruker Avance II NMR spectrometer (Bruker Biospin, Rheinstetten, Germany) with the following acquisition parameters: 350 ppm spectral width, 8 s relaxation delay, 128/2048 number of acquisitions and 64k data points. Optical imaging: Both fluorescence images and BLI images were acquired with an IVIS Spectrum system (PerkinElmer, Waltham, USA). For fluorescence imaging, epi-fluorescence illumination mode (FOV = 13.4 cm, Excitation peak = 648 nm, Emission peak = 670 nm) was used. D-luciferin (126 mg/kg) was administered intravenously after the cell/islets transplantation for BLI. MRI experiments: Both in vitro and in vivo MRI were performed using 9.4T Biospec small animal MRI system (Bruker, Ettlingen, Germany) equipped with a home-made surface coil tuneable and matchable to both ^{1}H MRI and ^{19}F MRI with same setups. RARE sequence was used for both in vitro and in vivo studies with some parameters modifications (RARE factor= 8/18; FOV= 6.4 cm*6.4 cm/8 cm*4 cm; TE= 15.9 ms; TR= 6 s (^{1}H)/1 s (^{19}F); slice thickness= 0.5 mm(^{1}H)/2.5 mm(^{19}F); Number of average= 2(^{1}H)/1000(^{19}F)).

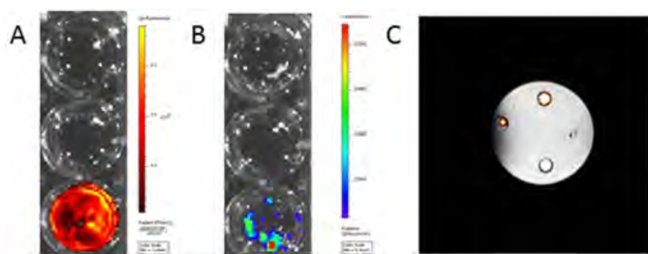

Fig.1 Representive in vitro images of three modalities
A. fluorescent image (upper-> bottom: unlabeled cells, PBS and PFCE labeled cells)
B. BLI image(upper -> bottom: un-transduced islets, PBS and transduced islets)
C. ^{19}F image(red hot scale) overlaid on ^{1}H image(gray scale) (upper->bottom : pure PFCE compound, PFCE labeled islets and PBS)

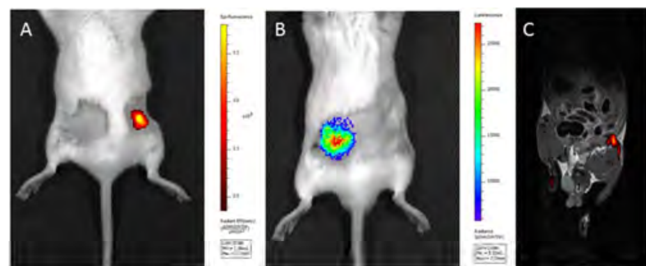

Fig.2 Representive in vivo images of three modalities
A. fluorescent image of transplanted PFCE labeled pancreatic islets
B. BLI image of transplanted LV transduced cells
C. ^{19}F image(red hot scale) of transplanted PFCE labeled islets overlaid on ^{1}H image(gray scale)

Results

The pancreatic islets/INS-1E cells were incubated with PFCE particles/lentiviral vector and then further used for in vitro and in vivo studies. Viability essays confirmed that PFCE particle and lentiviral vector did not affect cells/pancreatic islets' function and morphology. Microscopy images show both INS-1E cells and pancreatic islets have excellent uptake of PFCE particles after 24 hours' incubation. Confocal image confirms the internalization of the PFCE particles by some pancreatic cells. NMR results further confirm and quantify the uptake, which is around $4*10^{12}$ ^{19}F atoms/cell and $1*10^{15}$ ^{19}F atoms/pancreatic islet. In vitro BLI shows both INS-1E cells and pancreatic islets could be transduced successfully after a 48 hours' transduction. Phantoms containing labeled cells or pancreatic islets confirm good visualization by ^{19}F MRI (approx. 200 labeled islets) within an hour acquisition. After subcutaneous transplantation, cells/ islets could be detected and monitored in vivo by all three different imaging modalities, resulting in the following detectability thresholds: approx. 100 islets for ^{19}F MRI and 50 islets for both BLI and fluorescent imaging. Initial follow up results of PFCE labeled islets show the fluorescent signal could be tracked for over two weeks, while the ^{19}F MRI signal can be sustained for over 3 weeks.

Conclusion and Discussion

By using subcutaneous islet transplantation, we were able to cross-validate their location and quantity using a tri-modal in vivo imaging platform. All three different modalities could provide direct or indirect information regarding the number of engrafted pancreatic islets. Future work will focus on the follow up after the transplantation of islets in diabetic animals using all three imaging modalities. The pancreatic islet's tri-modal imaging has the potential to overcome the limitations in sensitivity, resolution and specificity of the individual methods.

References

1.Sakata N et al., Diabetes Metab Res Rev 30(1), 1-10(2014); 2. Ahrens ET et al., Nature Biotechnology 23, 983-987(2005); 3.Dewitte H et al., Journal of Controlled Release 169, 141-149(2013); 4.Wolfs E et al., Plos One 9, issue 4(2014).