In vivo imaging of islet transplantation in a mouse model of Type I Diabetes.

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Background

Type 1 Diabetes results from immune mediated destruction of pancreatic beta-cells, which leads to a deficiency in insulin secretion and as a result, to hyperglycemia. At present, pancreatic islet transplantation is emerging as the only clinical modality, which can stop diabetes progression without increasing the incidence of hypoglycemic events. Although early results of clinical trials are very encouraging, it is still unclear how long the islets will survive and how often the transplantation procedure will be successful. In order to monitor transplantation efficiency and graft survival, reliable non-invasive imaging methods are critically needed. If such methods introduced clinically, essential information regarding the location, function and viability of transplanted islets can be obtained repeatedly and non-invasively. This study describes in vivo imaging of transplanted human pancreatic islets labeled with iron oxide magnetic nanoparticles modified with Cy5.5 optical dye (MN-Cy5.5) for 4.5 month after transplantation in the mouse model of Type I Diabetes. Thorough investigation regarding islet function and viability upon labeling as well as nanoparticles distribution within islet cells is presented.

Materials and Methods

Human pancreatic islets were labeled with superparamagnetic iron oxide nanoparticles coated with crosslinked dextran, which was conjugated to Cy5.5 optical dye (MN-Cy5.5). Insulin secretion of labeled islets in response to glucose challenge was tested using ELISA kit (Mercodia, Uppsala, Sweden). Islet viability upon labeling was assessed with Trypan Blue stain. Distribution of MN-Cy5.5 probe within islet cells was tested using triple-channel fluorescence microscopy with anti-insulin, anti-somatostatin and anti-glucagon antibodies followed by a FITC-labeled secondary antibody in each case. For islet transplantation NOD.scid mice (n=5) were rendered diabetic by intraperitoneal injection of streptozotocin (STZ) 150 mg/kg. Animals with blood glucose level > 350 mg/dl were used for transplantation. Labeled human pancreatic islets were transplanted under the left kidney capsule. Non-labeled islets were transplanted under the right capsule and served as control. Repetitive in vivo MR imaging (up to 140 days) was performed in a 4.7 T GE magnet with a Bruker Biospin Avance console equipped with ParaVision 3.0.1 software. T2 maps were acquired using a transverse MSME sequence with the following parameters: TR = 2000 ms and multi-echo TE = 8, 16, 24, 32, 40, 48, 56, 64, NA = 4, RARE factor = 8, FoV = 4x4 cm^2, matrix size128 x 128, spatial resolution 312 x 312 μm^2 and slice thickness = 1mm. Correlative near-infrared optical imaging (NIRF) was performed two days after transplantation using whole-mouse imaging system (Imaging Station IS2000MM, Kodak), equipped with a band-pass filter at 630 nm and a long pass filter at 700 nm (Chroma Technology Corporation, Rockingham, VT). After imaging, the presence of MN-Cy5.5 probe in the islets was confirmed by immunohistochemistry.

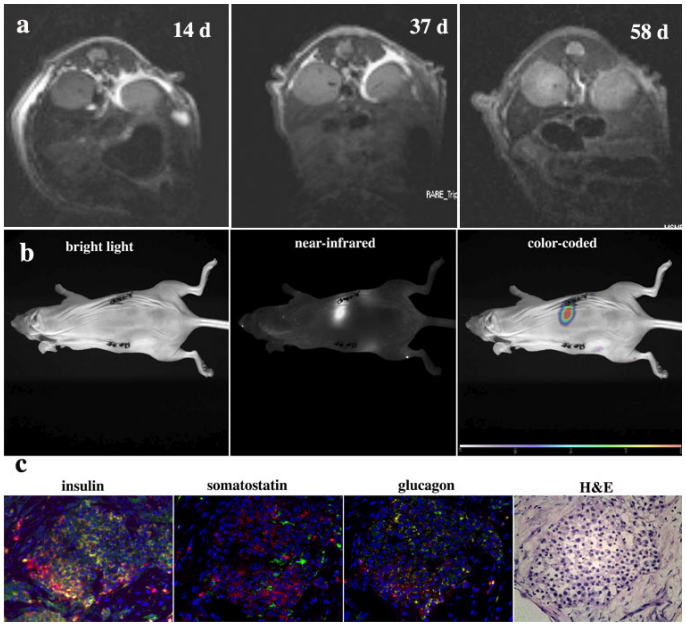

Figure 1

Results

Human pancreatic islet retained their function and viability after labeling with MN-Cy5.5. The distribution of MN-Cy5.5 probe was localized predominantly to beta-cells, and to much lesser degree to alfa- and delta-cells in the islets. Glucose-stimulated insulin secretion as seen by stimulation index was unchanged in labeled vs non-labeled islets (3.14 ± 0.58 vs 2.95 ± 0.78 respectively). In vivo imaging of mice transplanted with islets labeled with MN-Cy5.5 probe showed significant change in T2 in the left kidney compared to the right kidney (45%). Imaging was performed up to 60 days after transplantation (Fig. 1a). Mice started showing normoglycemia one week after transplantation. By 10 days all transplanted mice restored their normal glucose level. These results indicate that labeling of human pancreatic islets with MN-Cy5.5 probe did not alter their function in vivo. Optical imaging performed two days after transplantation revealed the bright NIRF signal coming from the transplantation site indicating the presence of labeled islets in the left kidney (Fig. 1b). These results confirmed our observations on MR images. The graft appeared labeled with MN-Cy5.5, which produced darkening on MRI images and NIRF signal on optical images. Two weeks after transplantation, some animals were sacrificed and their kidneys were removed for histology. Consecutive frozen kidney sections of the same islet were stained for insulin, glucagon and somatostatin (Fig. 1c). The results of fluorescence microscopy studies confirmed the presence of MN-Cy5.5 probe in the islets at the time of MR imaging. Furthermore, the distribution of the MN-NIRF probe within islet cells two weeks after transplantation correlated with that of the islets labeled in vitro. Most of the probe was associated with insulin-producing beta-cells, and to a much lesser degree with delta and alfa-cells.

Summary

This study demonstrates the plausibility of using superparamagnetic iron oxides for labeling human pancreatic islets. We concluded that islets could be labeled without altering their function or causing cytotoxic effects. Our study demonstrates longitudinal non-invasive monitoring of transplanted human islets in diabetic animals using magnetic resonance imaging. Restoration of normoglycemia was not altered by the label. Over all, our results indicate that it is possible to follow the presence and localization of transplanted human islets over time using iron oxides as a contrast media for islet labeling.