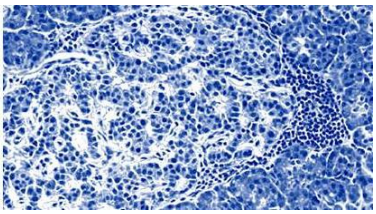
nPOD
Network for Pancreatic Organ Donors with Diabetes



Identification and Validation of a Novel 3D Imaging Technique for Accurate Quantitation of Human Beta Cell Mass in Islets Transplanted into Immunodeficient Mice

AUTHORS

Carolina Rosselot, Alexandra Alvarsson, Yansui Li, Rosemary Li, Peng Wang, Andrew F. Stewart, Sarah A. Stanley and Adolfo Garcia-Ocaña. From the Diabetes, Obesity and Metabolism Institute, The Icahn School of Medicine at Mount Sinai, New York, NY.

PURPOSE

Diabetes results from diminished functional beta cell mass. Recently, a number of small molecules, hormones and neurotransmitters have been identified that increase beta cell replication markers in vitro, and in human islets transplanted in immunodeficient mice in vivo. However, whether this increase in human beta cell proliferation markers translates into an increase in beta cell mass in vivo is controversial. Current beta cell mass determination methodology requires tedious sectioning, staining, multiple image reconstruction and analysis, and the challenge of creating whole graft 3D images and volume quantitation. Here, we use a tissue clarification technique called 3D Imaging of Solvent-Cleared Organs (iDISCO) and advanced light-sheet imaging tools to visualize and quantify the volume of human beta cells in islet grafts transplanted into immunodeficient mice.

METHODS

Human islets from adult healthy donors were transplanted under the kidney capsule of euglycemic immunodeficient B6.129S7-Rag1tm1Mon/J mice. Whole-mount staining and clearing was performed using the iDISCO method (Renier et al., Cell 2014). Z-stacked optical sections were acquired with an Ultramicroscope II at 1.3x or 4x magnification. Image analysis and calculation of beta cell volume was performed with Imaris software.

SUMMARY OF RESULTS

First, we transplanted 100, 300, 500 and 1000 human islet equivalents (IE, 1 IE=125µm diameter) under the kidney capsule of immunodeficient mice. Kidneys were harvested immediately after transplant to determine whether human accurate beta cell volumes could be calculated, whether there was a linearity between human beta cell volumes and the number of islets transplanted, and whether measured volumes of human beta cells correlated with the theoretical volume of transplanted human islets. Human beta cell volumes for the progressively larger mass of transplanted islets displayed almost perfect linearity ($r^2=0.9719$). As expected, human beta cell volumes were 20-30% lower than the theoretical volume of transplanted human islets, reflecting the abundance of non-beta cells in human islets. Second, we repeated the transplanted studies above, but harvested kidneys 14 days later. As expected, measured beta cell volume declined from 100% to 32±5% at

Day 14, likely reflecting ischemia-related beta cell death in the first days following transplant. Third, we transplanted 300 human IE in immunodeficient mice and treated the mice with harmine or vehicle for 3 months using Alzet minipumps. Preliminary results indicate that harmine treatment led to a three-fold increase in human beta cell volume over three months.

CONCLUSIONS

We have developed a robust, precise and highly quantifiable new tool to measure beta cell volume in human islet grafts. iDISCO is ideal for assessing long term changes in human beta cell mass in response to putative human beta cell proliferative agents. iDISCO can also be applied to accurately quantifying volumes of alpha, delta, PP, ductal, endothelial and other cell types in human islet cell grafts.