

## **SUPPLEMENTAL METHODS AND MATERIALS**

*Islet count.* Islet counts were performed on day 0 and day 1 after isolation. An aliquot was stained with diphenylthiocarbazone (dithizone or DTZ, Sigma, St. Louis, MO), and the number and size of islets were visually determined by the same operator. Islet volume was then extrapolated to calculate islet equivalents (IE), which standardizes the islet yield to a spherical islet of a 150  $\mu\text{m}$  diameter. All counts were done in triplicates.

*Islet purity and viability.* Islet purity was determined using dithizone staining done after islet isolation, and just before islet transplantation. A percentage value was assigned based on the tissue volume that stained with dithizone (islets) to the unstained tissue. Islet viability was determined by dual fluorescence staining with fluorescein diacetate and propidium iodide (Sigma, St. Louis, MO). A visual estimation of the green and red staining of islets  $>50$   $\mu\text{m}$  in diameter was made to determine the percentage of viable islets.

*Insulin secretion.* Labeled and unlabeled islets were incubated in static conditions at low and high glucose concentrations. The islets were washed 3 times in CMRL 1066 media (Cellgro; Mediatech, Herndon, VA), supplemented with 0.5% BSA, radioimmunoassay grade, 25 mmol/L HEPES, and 1.7 mmol/L glucose and pre-incubated for 60 minutes with this media. Basal insulin secretion was assessed by replacing the media with 2.5 mmol/L glucose for 60 minutes, followed by another 60 minute incubation with 16.7 mmol/L glucose. Supernatants were collected for both time-points and frozen for insulin assays. The islets were washed 3 times with PBS, and extracted with 0.18 N HCl in 70% ethanol for 24 hours at 4°C. The extracts were utilized to determine insulin content and for normalization of insulin secretion. Insulin concentration in the supernatants was

determined using an enzyme linked immunosorbent assay (ELISA) kit for human insulin (Merckodia, Uppsala, Sweden). Non-labeled islets served as controls. Each sample was done in triplicate.

*Islet harvest and autologous islet transplantation.* Partial pancreatectomy was performed through a midline laparotomy incision. The peritoneal cavity was accessed, and the retroperitoneum was exposed. The spleen was released off its attachments. The distal pancreas was dissected *en bloc* with the spleen. The pancreas was exposed proximally to the superior mesenteric vein – splenic vein confluence. The pancreas was cut anterior to the confluence to complete a 60% to 70% distal pancreatectomy. The distal pancreas was flushed on the back table with University of Wisconsin solution at 4°C, and transported to Joslin Diabetes Center (Boston, MA) for islet isolation. The remnant proximal pancreatic stump was oversewn, and the abdomen closed.

Post-islet isolation, the islets were cultured overnight, and transplanted into the baboon the next day. The first baboon received an autologous islet transplant: 75% of the islets were transplanted underneath the renal capsule to prepare an islet-kidney (IK), and 25% of the islets were infused into the liver. A small left flank incision was used to expose the left kidney, and using a 22 gauge catheter the islets were injected underneath the renal capsule. The flank incision was closed, and then a small right lower quadrant incision was made to access the ileocecum and its mesentery. The ileocecal vein was cannulated, and islets were infused into the liver. The incision was closed, and the animal was recovered.

In the second baboon partial pancreatectomy was performed in the same way as the first animal. The next day, autologous islet transplant was done, with 75% islets infused into the liver and 25% of the islets used to prepare a left islet-kidney.

*Completion pancreatectomy.* After >6 months of follow-up a completion pancreatectomy was performed to remove the remnant proximal pancreas. The pancreatic head and the uncinate process were dissected en bloc with the duodenum. Continuity of the GI tract was achieved through a gastro-jejunostomy and a choledocho-jejunostomy. The animal was followed to test if Feridex-labeled islets could regulate glucose in vivo.

*Animal handling during imaging.* The baboon was fasted for 8 hours before MRI. Thirty minutes before sedation, atropine was given intramuscularly (IM) at 0.05 mg/kg.

Ketamine was given IM at 5 mg/kg for sedation. In the operating room, a peripheral venous catheter was placed for intravenous access, and propofol infusion was started.

The animal was intubated with a #5 endotracheal tube for airway protection and transported to the MRI suite once normal vital signs were confirmed under sedation. It was placed supine on the MRI table, and its extremities were secured to the table with tape. The endotracheal tube was connected to the respiratory monitor, and continuous 2L/min oxygen flow was given. Electrocardiogram leads were also placed for continuous cardiac monitoring. During the MRI, the animal was maintained on propofol sedation, and continuously monitored for respiratory rate, oxygen saturation, and end tidal CO<sub>2</sub>.

Propofol dosing was titrated accordingly based on vital sign parameters. In order to minimize variability in animal positioning, during every imaging session the body matrix coil was placed over the trunk, covering the area from the areola to the pubic symphysis. An initial localizer scan was performed for optimal slice positioning. Slices were

positioned so that the middle slice was located 2 to 3 cm above the branching of the portal vein (liver) or the center of the graft covering the entire graft volume (kidney).

Imaging parameters were as follows:

Liver:

- a) (with respiratory gating) - TR/TE = 100/2.3-29.3 ms, slice thickness 3 mm, FoV = 200 x 200 mm<sup>2</sup>, matrix size 192 x 192, flip angle = 25°, number of slices = 10, number of averages = 1, and in plane resolution 1 x 1 mm<sup>2</sup>.
- b) (with navigator) - TR/TE = 2000/4.76-61.3 ms, slice thickness 3 mm, FoV = 200 x 200 mm<sup>2</sup>, matrix size 256 x 256, flip angle = 70°, number of slices = 12, number of averages = 1, and in plane resolution 0.78 x 0.78 mm<sup>2</sup>.

Kidney:

- a) (with respiratory gating) - TR/TE=200/2.1-29.1 ms, slice thickness 3 mm, FoV = 180 x 180 mm<sup>2</sup>, matrix size 192 x 192, flip angle = 25°, number of slices 12, number of averages = 4, and in plane resolution 0.9 x 0.9 mm<sup>2</sup>.
- b) (with navigator) - TR/TE = 2000/4.76-61.3 ms, slice thickness 3 mm, FoV = 180 x 180 mm<sup>2</sup>, matrix size 256 x 256, flip angle = 70°, number of slices = 12, number of averages = 1, and in plane resolution 0.7 x 0.7 mm<sup>2</sup>.

### *Image Analysis*

Image analysis was performed using the FreeSurfer 3.0.4 interactive graphical user interface (GUI), Scuba (9; 10). The analysis of transplanted islet mass was predicated on manual delineation by a blinded observer of a region of interest (ROI) defined by the boundary of the kidney or the liver in all slices of the image. As such, this region contained signal stemming from two sources: kidney/liver parenchyma, characterized by

high signal homogeneity, and transplanted islets, characterized by distinct drop in  $T2^*$  values.  $T2^*$  maps were constructed taking into account all of the echoes acquired during imaging. Once these volumetric labels were available, we used an automated segmentation method K-Means (based on the MatLab implementation) to classify the ROI into two labels based on  $T2^*$  values: the graft label and the kidney/liver parenchyma label. The final outcome was a first-order estimate of the transplanted islet mass. Data were represented as number of voxels occupied by the graft as a function of time.