Following of the Fate of Transplanted Pancreatic Islets in the Early Post-transplant Period by MRI. Their Automatic Detection and Quantification.

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Introduction

The visualization of iron labeled cells *in vivo* with experimental MRI has become routine. However, it is not always possible to identify the iron labeled cells unambiguously and quantification is not straightforward. Here we present a novel transplantation model and a simple and robust segmentation method, which allow an easy quantification of labeled cells. Our approach was applied to the task of monitoring the fate of transplanted pancreatic islets (PI) in diabetic mice during the early post transplant period.

Subjects and Methods

PI were isolated using collagenase digestion followed by Ficoll® gradient purification from C57BL/6 and BALB/c mice. The isolated pancreatic islets were stained in CMRL-1066 medium (HyClone, USA) supplemented with the superparamagnetic contrast agent Feridext® (5µg Fe/ml, Berlex) complexed with poly-L-lysine overnight. After removal from tissue culture, the 200-230 PI (syngeneic transplantation ST, n = 3; allogeneic transplantation AT, n=4) were washed out and then slowly injected into the right liver lobes of BALB/c diabetic mice (Streptozocine i.p., 220 mg/kg). Blood glucose levels were measured every other day after transplantation. To verify segmentation technique, 1-10 PI were placed in a single plane sandwiched gelatin phantom.

Anesthetized animals were scanned on days 1, 3, 5, 7, 10 and 14 after PI transplantation (Tx). Imaging was performed on a 3T GE MR scanner using a custom-built gradient coil and a customized whole-body solenoid radiofrequency. Animals were imaged using a 3-D fully refocused (steady-state free precession) gradient-echo sequence with a resolution of 78x78x100 μm^3.

MR images were processed using a custom-made program in Matlab (MathWorks,USA), which detects hypointense regions representing PI independently of the user. It is based on morphological top-hat and bottom-hat transforms [1]. All pixels considered by the program as PI in the phantoms or liver tissue were set up as black pixels (signal intensity SI = 0) and counted using ImageJ software (NIH, USA). Total signal loss area (SI = 0) detected on the MR images within the liver tissue 1 day after transplantation was rated as 100% and subsequent measurements of signal loss regions were recalculated as relative numbers.

Results

Automatic segmentation was at first applied to MR images of gel phantoms with labeled islets, which were observed as hypointense areas (Figure 1A). After data processing, the total automatically segmented area (Figure 1B) was 95 ± 6% of the manually measured area.

Regions of signal loss attributed to transplanted islets were detected in MR images of the right liver lobes, confirming the technical success of Tx. No signal loss was observed in the non-transplanted lobes or in the livers of control animals (Figure 2). There was no statistically significant difference in the total area of signal loss between the groups AT and ST (T-test, $p<0.05$) in the first and second weeks. During the first week we observed a dramatic decrease in the regions of signal void (up to 56%) in both transplant groups (AT, ST), however there was a significant difference in weekly decrease of signal loss second week following the transplantation (T-test, $p<0.05$). These measurements are summarized in Table 1.

Glycemia was normalized within 3 days after Tx. While isograft recipients remained normoglycemic until the end of experiment, allograft function failed between 5 to 10 days after Tx. The basic histological examination showed a well-preserved structure of transplanted PI isografts without any lymphoid infiltration. In contrast, all stages of islet destruction and lymphoid infiltration were observed within the livers of allograft recipients.

Discussion and Conclusions

MRI analysis supported the hypothesis that in the first few days repeatedly described as high-risk period for islet engraftment there is erratic response to transplanted tissue while in the second week the response is steady and difference of signal loss decrease in isografts and allografts Tx was observed due to the effect of acute rejection [2]. The presence of transplanted PI in the liver's right lobes and their absence in control counterpart lobes allowed for the optimization of an automatic segmentation technique in this novel Tx model. Thus, the quantification is more reproducible and minimizes the subjective evaluation.

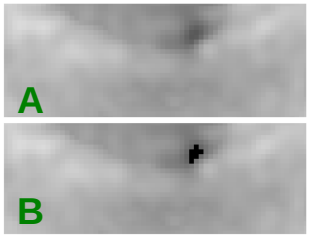

Figure 1: MR image of labeled pancreatic islets in gelatin phantom acquired with the same resolution as was used for in vivo experiment (voxel size 78 µm x 78 µm x 200 µm) (**A**); the same MR image overlapped with binary image with automatically detected area (black pixels) (**B**).

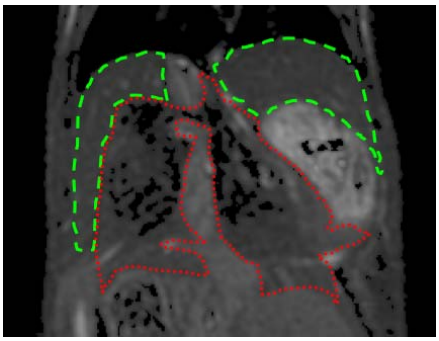

Figure 2: *In vivo* MRI of a mouse liver one day after allogeneic transplantation into the right lobe (red dashed line). Islets were detected automatically. No islets were detected in control liver tissue (green dotted line).

Animal group	Relative signal loss [%]					
	Days after transplantation					
	1	3	5	7	10	14
AT	100	85.1±8.8	71.8±7.0	62.4±8.8	45.5±9.2	36.2±4.3
ST	100	81.1±17.4	67.3±15.0	51.7±12.4	48.6±11.8	40.6±12.5

Table 1. Relative area of signal loss for automatically segmented PI during the early post-transplant period in two Tx groups (AT, ST). A statistically significant decrease in the area of signal loss compared to initial values was observed at 3 days after transplantation in AT and 5 days after transplantation in ST (T-test, $p<0.05$).

References

1. Russ JC, The Image Processing Handbook, Fifth Edition (Image Processing Handbook), CRC 2006.
2. Kriz J et al. Magnetic resonance imaging of pancreatic islets in tolerance and rejection. *Transplantation*. 2005.

Acknowledgement

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