Assessment of Pancreatic Islet Grafts Survival by Immunostaining

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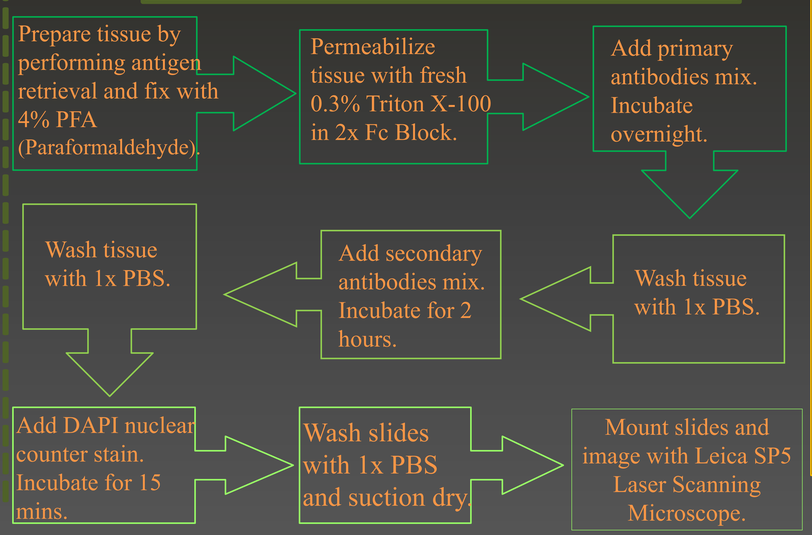
1 Introduction

Type 1 Diabetes (T1D) occurs when the pancreas is unable to produce insulin and regulate the glucose level in the blood. Transplantation of the insulin producing β-cells of the islets of Langerhans in the anterior chamber of the eye (ACE) has proven to be an advancement in the treatment of T1D. A crucial advantage in islet transplant in the ACE is being able to track the islet grafts noninvasively and longitudinally and detect immune system attack against them before their complete destruction for timely intervention. The objective of this study was to determine whether immunostaining is a reliable technique to further assess the survival of the transplanted islets in non-obese diabetic (NOD) mice prone to T1D. Immunostaining consisted of layering mixes of primary and secondary antibodies to the transplanted mice eye sections on slides for analysis under a Leica SP5 Laser Scanning Microscope.

2 Methods



3 Results

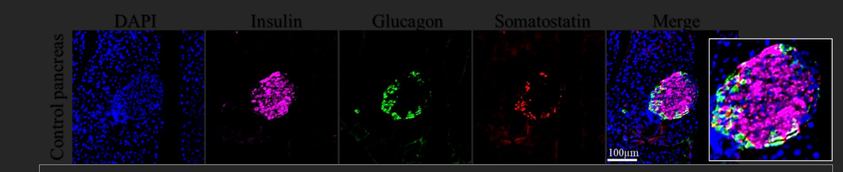


Figure 1: **Immunostaining performed in control mouse pancreas tissue not subject to immune attack**. For the islet shown, DAPI solution was used as a nuclear counter stain, insulin β-cells are presented in magenta, glucagon α-cells in green and somatostatin δ-cells in red. A merge of the pancreatic islet cells shows the composition of the islet: β-cells in the center, α-cells in the periphery and δ-cells scattered around the islet.

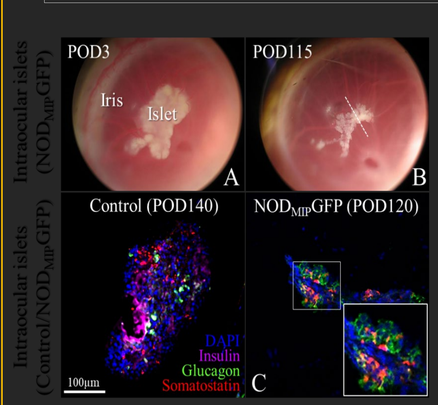


Figure 2: **Immunostaining in NOD$_{MIP}$GFP islets transplanted in the ACE of NOD mice.** *In vivo* images from a NOD mouse that was transplanted with NOD$_{MIP}$GFP islets in the ACE acquired on POD3 (A) and POD115 (B) show development of the immune destruction against them. Immunostaining (C) confirmed the identity of the remaining islet cells as glucagon (alpha) and somatostatin (delta) positive cells following the immune attack on the insulin positive (beta) cells. The dashed line on the POD115 image represents the section of the islet that was immunostained.

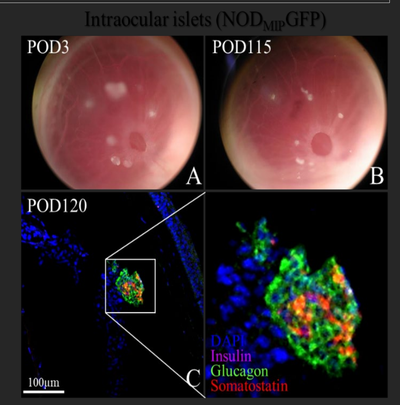


Figure 3: Immunostaining performed to NOD$_{MIP}$GFP mouse eye tissue. *In vivo* images from an NOD$_{MIP}$GFP acquired POD3 (A) and POD115 (B) show the development of the intraocular islet graft. The reduction of the islet size is noticeable from panel A to B. The stained islet (C) shows the DAPI counterstained nuclei in blue, glucagon α-cells in green and somatostatin δ-cells in red. Insulin β-cells that would appear in magenta are not present. This confirmed the attack and destruction of the beta cells and that the remnant cells composing the islet were α- and δ- cells.

4 Conclusion

The fluorescence immunostaining technique employed here resulted in efficient identification of the endocrine cells within islet grafts subjected to immune attack following transplantation in the ACE of NOD recipient mice. Importantly, this method complemented the *in vivo* studies by identifying the cells that remained within the islet grafts following the immune attack. The *in vivo* studies showed the progressive decrease in the volume of the islet grafts, but immunostaining confirmed that this was due to β-cells immune destruction rather than that of alpha and delta cells. In summary, although restricted to tissues that can only be acquired post euthanasia, the current study demonstrated that fluorescence immunostaining can contribute critical information about islet graft survival and cell identity.

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5 Acknowledgements

DIABETES RESEARCH INSTITUTE FOUNDATION
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