Engraftment of a Functional and Vascularized Pancreatic Organoid By Random Assembly of Micro-tissue Units

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Introduction:

The delivery of insulin-secreting cells, such as human embryonic stem cell (hESC) -derived pancreatic progenitors, offers a promising treatment for insulin-dependent Type 1 diabetes. However, survival and long-term function of therapeutic cells upon implantation in the host are significant barriers to clinical translation, largely due to poor vascularization of the graft. Pancreatic islets receive 10% of the organ's blood supply, despite comprising merely 1% of the pancreas' mass. An intricate vascular structure, in which there is one-to-one contact between beta-cells and endothelial cells of blood vessels, is crucial to survival and endocrine function. My project is framed upon the premise that recapitulating the islet's vascular architecture will improve long-term engraftment of insulin-secreting cells, thereby necessitating smaller cell dosages for the reversal of Type 1 diabetes.

Modular tissue engineering is a strategy to construct tissues with integral vasculature. Cylindrical collagen pieces, known as modules (1 mm length and 400 micrometer diameter), contain adipose-derived mesenchymal stromal cells (adMSCs) and are enveloped by endothelial cells (ECs). When injected subcutaneously, modules assemble randomly and, through a process of remodeling *in vivo*, create a perfusable vasculature. It is hypothesized that the vasculature established using modular tissue engineering will enhance survival of hESC-derived pancreatic progenitors in the subcutaneous space by providing an oxygenated microenvironment, and through paracrine factors produced by ECs and adMSCs. This effect is expected to be further enhanced within small (200 micrometer) diameter modules, in which pancreatic progenitors are more intimately associated with ECs, adMSCs and vascular networks. The objectives of the study are to 1) assess engraftment of pancreatic progenitors in 400 micrometer diameter modular tissues containing adMSCs and ECs, and 2) assess their engraftment within 200 micrometer diameter modular constructs containing adMSCs and ECs.

Materials and Methods:

Modular tissue constructs comprised of angiogenic (EC-coated adMSC-embedded) modules, and of pancreatic progenitor-embedded modules, were transplanted subcutaneously into the dorsum of SCID-bg mice (n=3). Modules containedg functional cells in type I collagen at 1×10^6 adMSCs/mL or 8×10^6 pancreatic progenitors/mL, and coated with human umbilical vein ECs (HUVECs). Modules were injected subcutaneously into the dorsal region of SCID-Bg mice, and explanted at 7 and 9 weeks post-transplantation for immunohistochemical analysis.

In a subsequent experiment, angiogenic modules of two different diameters (200 and 400 micrometers) were implanted to assess the effect of module size on the angiogenic response *in vivo*. All modular implants were comprised of the same volume of collagen (0.25 mL type 1 rat tail

collagen), and number of adMSCs (2.5 $\times 10^5$ adMSCs at a cell density of 1×10^6 adMSCs/mL). Modular implants were injected subcutaneously in the dorsum of SCID-Bg mice and explanted at day 3 and 7 post-implantation for immunohistochemical analyses. All animal surgeries were performed with the approval of the University of Toronto Animal Care Committee.

Results:

In a few pancreatic progenitor and adMSC-EC implants, pancreatic progenitors were found in close association with EC networks. Within these aggregates, some progenitor cells matured into insulin-producing cells.

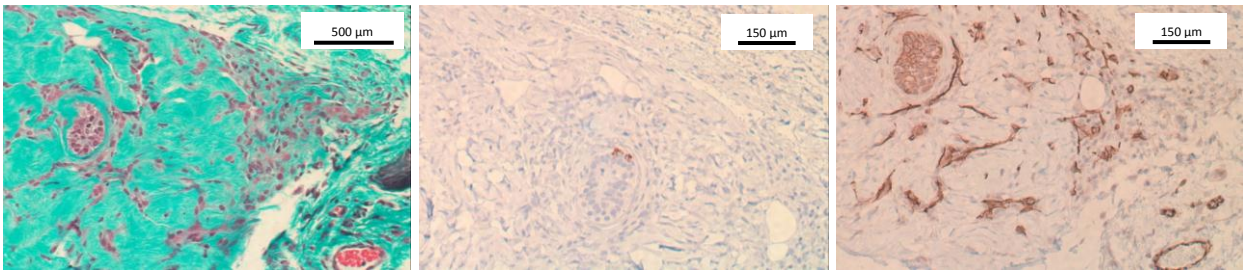

Figure 1. A modular implant harvested 7 weeks post-implantation containing pancreatic progenitors and adMSC-EC modules, serially stained for trichrome (left), insulin (center) and CD31 (right). The same pancreatic progenitor aggregate is highlighted within the red box.

Miniaturizing angiogenic (adMSC-EC) modules from a 400 to 200 micrometer diameter resulted in the formation of vascular networks that were closely associated with the center of modules, where functional insulin-secreting cells are to be embedded.

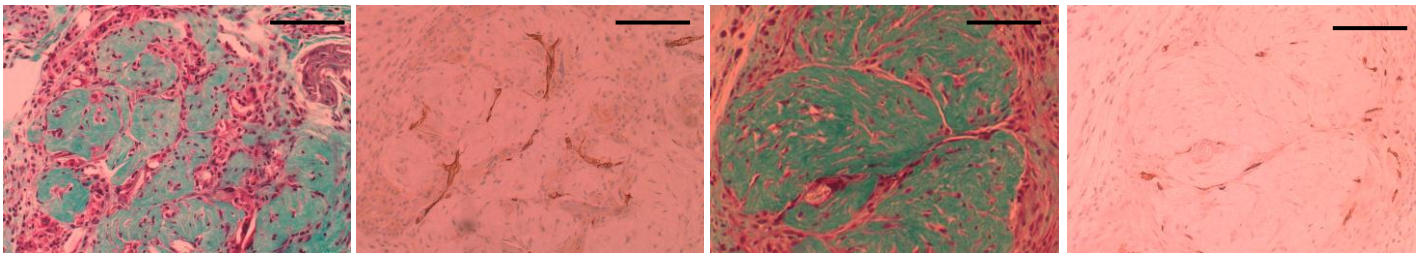

Figure 2. Trichrome (A, B) and CD31 (C, D) staining of 200 micron diameter (A, B) and 400 micron diameter modules explanted at day 7 post-implantation.

Discussion:

Preliminary results indicate that a challenge associated with the conventional sized modules (400 micrometer diameter) is localization of pancreatic progenitors and vascular networks within the implant. With the exception of a few animals, immunohistochemical analyses of most implants at 7 and 9 weeks post-implantation show that pancreatic progenitors are distant from vascular structures. This presumably impinges on proper engraftment.

To address this limitation, I have begun to investigate the effect of module size on the angiogenic effect of modular tissues. Miniaturizing modules to the 200 micrometer diameter range results in the development of vascular networks in close associated with the center of modules. The next step of this study is to investigate whether implantation of insulin-secreting cells in small-diameter modules will enhance engraftment in comparison to large-diameter modules.