The Islet Confidential: Recent Trends and Perspectives in Pancreatic Islet Transplantation

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ABSTRACT: Diabetes ranks among the top 5 killer diseases of the current world population. Transplantation of pancreatic islets is a common surgical procedure used to combat the late stage diabetic complications. A successful and long lasting islet transplant is an enigma as the complex immunoactivation mechanisms against the transplants, the subsequent graft rejection and the proper maturation and functioning of the islets in the host microenvironment, are the subjects of research for many years. This review details certain recent studies performed upon primate, porcine, murine and rabbit models, in relation to islet transplantation, with a critical standpoint.

1. INTRODUCTION:
Diabetes is a complex disease with various sub-classifications, characterized primarily by insulin dependency and multiple organ damage. Diabetic etiology is very extensive involving family genetics, life style, food habits, environment and microbial infections.
In humans, glucose metabolism is primarily controlled by insulin secretion by the endocrinal pancreatic β cells. In spite of their physiological significance, the β cells are one of the most endangered and least equipped cells, as they are much vulnerable to the attacks by free radicals, metabolic and endoplasmic reticular stress and inflammatory cytokines [1-4].
Pancreatic islet transplantation is one of the treatment strategies for diabetes. The Edmonton protocol for islet transplantation [5] pioneered numerous subsequent studies on islet transplantation. Following this study begun the long list of reports focusing upon trends such as usage of inert crosslinking materials, molecules for islet survival and graft rejection mechanisms, in order to achieve transplantations optimal. Some of these trends are detailed below.

2. IMMUNOACTIVATION:
The activation of immune system and the subsequent coup by tissue specific cytotoxic T lymphocytes, T½ cells, antibodies and the safety measures following any immunosuppression are serious problems to be considered. Various studies are reported regarding the immunoactivation, and suppression mechanisms involved in the onset of diabetes, graft rejection and stability.
Studies report the association of certain polymorphisms of the genes IL-17E, ATF-6, HNF4A, PRDM14 [6] and mannose binding lectin (MBL) gene [7] to be associated with the development of diabetes in transplantation. Certain non-MHC regions on chromosomes 1, 4, 6 and 9 also are reported in porcine model of immunologically mediated corneal graft rejection [8].
Cornelis R. van der Torren et al. in their interesting study, report the generation of β cell lines EndoC-βH1 and ECi50 to study both the innate and adaptive immune processes involved in the β cell destruction In-Situ, both in diabetes and transplantation. Lentiviral induction of HLA A*02:01 upon EndoC-βH1 cells was achieved. Transduction with exogenous Cytomegalo virus (CMV) peptide epitope also was performed. The cell lines were cultured with TH1 cells and incubated with inflammatory cytokines. Hence, this study meticulously simulated the cellular and molecular conditions a β cell could encounter.

As a result, the HLA class 1 and class 2 expressions were profiled and the EndoC-βH1 cells were typed to be HLA A*33:03 and HLA A*68:01 and the ECi50 cells to be HLA A*02:02 and HLA A*68:01. Significant increase of β cell death was observed in both cell lines. Incubation with PPI (Pre-pro-insulin)-specific cytotoxic T lymphocyte (CTL) increased the cytolysis. Cells transduced with CMV epitope were killed by CMV-specific CTLs. Cells with HLA A*02:02, if upregulated by IFNγ were killed by alloreactive T cells. Higher HLA expression also resulted in lysis of cells by alloreactive antibodies, especially upon cells with HLAs upregulated by IFNγ. Cells expressing low HLAs were, in turn, killed by activated natural killer (NK) cells. The cells expressed complimentary inhibitory receptors, such as CD59 and CD46 and escaped the killing by complements. This work elucidated various immunoactivation mechanisms associated with β cell death. Xiaohai Zhang et al. [10] report the role of HLA class 1 antibodies in immunoactivation by binding with antibodies and the cascade of leukocyte recruitment.

Similar to these works is the study by Ines G. Harper et al. [11] which suggest a major role of donor lymphocytes in alloimmunity against the heart transplants in murine model. They have generated a bm12-C57BL/6 mice model with additional MHC class 1 and class 2 molecules. bm12-specific passenger CD4T cells initiated the production of antibodies. No complement C4d deposition was observed in T cell deficient (Tcrbd−) mice (Figure.1).

Nan Wang et al., [12], in their extensive study with bone marrow derived haematopoietic stem cells (BM-HSCs) in murine model detail various factors which could lead to immunoactivation and subsequent diabetes.

In their study, they report higher number of conventional T cells (Tcon cells) and lower Treg cells in pancreatic lymph nodes (PLNs) of older recipients. The proliferation rates of Tcon cells of PLNs, blood and mesenteric lymph nodes (MLNs) in younger recipients were observed to be lower. Significant reduction of Thymic Tcon cells also was observed in younger recipients. This study suggests that older recipients could develop diabetes more rapidly. Hyperthyroidism also is reported, in rabbits, to induce immunoactivation which could, in turn, lead to infiltration of immune cells [13].

Indoleamine 2,3-dioxygenase (IDO) [14] has been reported to be involved in the suppression of immunoactivated cells. Inhibition of Cyclin dependent kinase-9 (CDK-9) by PHA767491 also could protect the graft from alloreactive CD4T cells [15].

Nadine Nagy et al., [16], in their study with DORMO and NOD murine models reports that deposition of hyaluronan (HA) could be implicated in the immunoactivation as it lead to the development of autoimmune diabetes. Inhibition of HA synthesis by 4-methylumbelliferone (4-MU) prevented various complications of diabetes. Similar view was reflected in the work by
Marika Bogdani et al. [17] in their work with diabetic tissue donors in the Network for pancreatic organ donors with diabetes (nPOD) program. The same group [18]) reports the detection of glycosaminoglycan hyaluronan and heparin sulphate in pancreatic β cells. They achieved it using an hyaluronan binding protein (HABP) specific probe.

3. MICROBIAL INFECTION:
Suresh Paudel et al.,[19], in their meta-analyses of reports of subjects received solid organ transplantation (SOT) during the period of 1991 to 2014 indicate that there is a high prevalence of Clostridium difficile infections (CDI) among the transplant recipients. The prevalence of CDI infection among the pancreas recipients is determined to be 3.2% [95% CI, (0.5%-7.9%)].This analyses necessitates proper interventions to combat CDI.

Sung hang kim et al., [20], in their study with kidney transplants suggest that early diagnosis for CMV specific T cells could effectively predict post transplantation CMV infections.
In their study with North american child solid organ transplant (SOT) recipients, Paul K. Sue et al., [21], reports that hepatitis E virus (HEV) is the major infection which could lead to graft rejection of SOT. Marcio F. Chedid et al. [22] reports the significance of hepatitis C virus (HCV) infections in the elicitation of hepatocellular carcinoma in liver transplants. This study recommends post transplantation strategies such as transarterial embolization and ethanol injection to combat this dilemma. In addition to HEV, HCV infections also are implicated in graft rejection in liver transplantation [23].

4. ENCAPSULATION:
Various encapsulation approaches are devised to insulate the grafts and stem cells from immune cells and enable the proper adaptation and functioning of the transplants in the host microenvironment.
Alan D. Agulnick et al. [24] report the use of VC-01,a macroencapsulation device for the In- vivo transfer and differentiation of human embryonic stem cells (hESCs).These cells effectively differentiated into islet-like cells (ICs) as observed by PDX1⁺ and NKX6.1⁺ cells (Figure.2).

Andrew R. Pepper et al.[25] reports the use of Sernova Corp's Cell Pouch (CP), subcutaneous cell pouch. The islets were capsulated in CP and transplanted in diabetic mice. CP- encapsulated BALB/c mice responded well to glucose challenge and exhibited enhanced positivity for both insulin and glucagon (Figure.3).
Woon Teck Yap et al. [26] suggest the use of modified collagen scaffolds for islet transplantation. Another similar work [27] suggests the use of collagen-chitosan hydrogel for islet transplantation. Friederike Ehrhart et al. [28] report the use of multilayered alginate coatings for the encapsulation. The islets were reported to be biocompatible with optimal insulin secretion.

Naturally occurring compounds such as fibrin [29], Amylin [30] and laminin also were reported [31] to be used as effective scaffolds for islet encapsulation. The usages of scaffolds for various tissue types are reported [32]. Interestingly, scaffold-free stem cell constructs also are recommended for osteoinduction [33].

The significance of surrounding matrix environments were considerably reported [17, 34] in their relation with islet function and survival. Ulrika Johansson et al. [35] recommend the usage of spider silk matrices for effective transplantation and long term survival of β cells.

In their study, three forms (Fiber, foam and film) of silk were used. The cell binding motif RGD was clonally inserted to the silk matrices. The β cells were transplanted into the anterior chamber of the eye to enable in vivo imaging of islets. As result, the cells kept in foam form exhibited significant revascularization (Figure 4).

**FIGURE 4:** Mouse pancreatic islets adhere to silk matrices (A) Photographs of the various formats; fiber (left), foam (middle) and film (right) and micrographs together with adhered islet (lower panel, asterisks). Scale bars = 50μm. (B) Micrographs of islets after 2 weeks in control wells (left), on WT foam (middle) and on RGD foam (right). Scale bars = 50 μm. (C) Morphology by H/E (left panel) and insulin (green, right panel) staining of eye sections showing representative (n = 3) control islet (upper graphs) and islet from RGD foam (lower graphs). Vasculature was seen in islets from both culture conditions (white arrowhead) although vessels with erythrocytes were more common in islets from RGD foam. Areas of visual cell death were sometimes present in control islet (white lined circle). Scale bars = 50 μm. (Adopted from Ulrika Johansson et al.).
Di Wu et al. [36] reports the MIN-6 β cells embedded in engineered 3D-decellularized extra cellular matrix (ECM) scaffold could effectively simulate tissue microenvironment. Pancreas were harvested from C57BL/6J mice. Decellularization was achieved by removing perfusate using peristaltic pump, washing by distilled water and subsequent storage by -80°C. Later the cells were washed by PBS and Triton X. Vasculature was measured by Angiography. The scaffold was placed subcutaneously. Eosin–Hematoxilin (HE) and immunofluorescence staining was performed on the scaffold. Finally, the MIN-6 β cells were infused into the scaffold.

The recellularization of the engrafted cells was confirmed by SEM imaging. Expression of the Insulin gene of the grafted cells was sufficiently higher (Figure. 5). Intriguingly, one of the test groups of the scaffolded grafts indicated higher blood glucose levels. It was reasoned in this study, that it could be the result of a lack of nutrition supply.
5. CROSSLINKING:

Crosslinking is another process utilized to prevent any elicitation of immune reactions against the graft. An acellular tissue environment could provide a compatible and safe environment for tissue growth and repair [37, 38]. One of such crosslinking agents commonly used was Glutaraldehyde (GA) [39]. But GA is limited by its immunogenicity. In contrast, Saeromi Jeong et al. [40] suggest the better cross-linking property of GA. Other reports suggest Genipin, a naturally occurring crosslinking agent to replace GA and for an optimal scaffolding [41-43]. Recently, Yujia Wang et al. [44] in their study with porcine liver transplants suggest that Genipin could significantly reduce the immunogenicity compared with GA (Figure. 6).

Various nanotechnology based approaches has been suggested for better biomolecular conjugation. Andreas M. Nyström et al.[45] reports the conjugation of Thiol-functionalized shell crosslinked knedel-like (SCK) nanoparticles with bovine serum albumin (BSA). I. M. El-Sherbiny et al.[46] suggest the copolymerization of polyethylene glycol(PEG) with N-phthaloyl chitosan (NPHCs) to be used as respirable alginate hydrogel microspheres for an optimal pulmonary drug delivery. Alginate hydrogels are also reported to be effective crosslinking drug carriers in stomach [47].
FIGURE 6: (a) Macroscopic appearance of native liver, (b) decellularized liver extra cellular matrix (ECM), (c) genipin-fixed ECM and (d) GA-fixed ECM. (e-h) Histological images of liver samples stained with H&E after dehydration and sterilization processes. Scale bars = 100 μm. (i-l) Scanning electron microscopy of the liver materials. (Adopted from Yujia Wang et al.).

Tania Betancourt et al. [48] recommend pH-responsive hydrogels of poly-itaconic acid-g-ethylene glycol for oral drug delivery. Recently, Qinmei Wang et al. [49] reports an injectable hydrogel composed of oxidized alginate(OA) crosslinking gelatin with electroactive tetra-aniline -graft OA-nanoparticles (nEOAs). Hydrogel formations with hyaluronate [50], nanofibers [51] are reported to be effective for bone regeneration and arterial scaffolding, respectively. Hyaluronan scaffolds are also reported to be involved in smooth muscle cell (SMC) regeneration [52].

6. VASCULARIZATION:

Vascularization signifies the preliminary adaptation of the cells into the molecular niche after transplantation. The formation of blood vessels ensures the traffic of nutrients, growth and survival of the cells. Thyroidectomy- induced devascularization is reported to cause islet infiltration in rabbits [53].

Cara E Ellis et al. [54] reports an artificial vascularization matrix made up of collagen crosslinked with 1-ethyl-3- carbodiimide and N-hydroxysuccinimide and chondroitin-6-sulfate, chitosan, and laminin. This study was performed with neonatal porcine islets (NPIs) transplanted subcutaneously upon immunocompetent B6.Rag mice. The combination of the co-polymers ensured the function, survival and vascularization of the islets post-transplantation (Figure. 7).
FIGURE 7: Right: Dark field images of NPIs embedded in Collagen alone (C) (A) Collagen-Chitosen (CC) (B), Collagen-Chitosen-Chondroitin (CCC) (C) and Collagen-Chitosen-Chondroitin –Laminin (CCCL) (D) after the matrices were cultured for 7 days (Scale bars: 400 μm for (A) and (B) and 1.6 mm for (C and D). Left: Morphology of NPIs in the CCCL gels and transplanted in immunocomprised B6.Rag −/− mice. Circles indicate visible islets; arrows indication visible vasculature of pre-transplant (A) and after 4 (B), 21 (C) and 28 (D) days. Scale bars are 400 μm (Adopted from Cara E Ellis et al.).

Similar to this study is the recent report by Edward A. Phelps et al.[55] which suggest the use of polyethylene glycol maleimide (PEG-MAL) hydrogels for the encapsulation of islets. The grafted cells functioned properly and secreted insulin. This addition of vascular endothelial growth factor (VEGF), the primary molecular of vascularity, resulted in optimal vascularization of the grafted cells (Figure. 8). VEGF is also reported to cause lymphoangiogenesis, in corneal allogeneic transplantations on murine model [56].

FIGURE 8: Islet transplantation with PEG-MAL and alginate. A Hydrogels were cross-linked directly onto the tissue surface of the mesentery. B Macroscopic images of implant site at 0, 1, and 4 weeks. C Whole mount immunostain for insulin of explanted hydrogel at 4 weeks. D Quantification of islets in immunostained explants. (Adopted from Edward A. Phelps et al.).
7. OTHER BENEFICIAL FACTORS:

Nathalie M. Fiaschi-Taesch et al. [57] in their study with non human primate (NHP) islets, reports that hepatocyte growth factor (HGF) could enhance the survival rate of islets post-transplantation (Figure 9).

![Figure 9: Photomicrographs of adenovial transduction of 500 NHP islet equivalents (IEs) with murine HGF. (Adopted from Nathalie M. Fiaschi-Taesch et al.)]

Another complementary study [58] with porcine islets suggests the property of adenosine in preserving pancreas for islet transplantation (Figure 10).

![Figure 10: DTZ staining of islet yields with different perfusates. A. Control group with University of Wisconsin (UW) perfusion. B. Experimental group with UW added with adenosine perfusion solution (Adopted from W.Q. Song et al.).]

Other molecules such as anti-CD40 antibody [59] and Ribonuclease (RNase) [60] also are reported to be associated with graft survival in heart transplantation model.

8. ALTERNATIVE TRANSPLANTATION ROUTES:

Morteza Abouzaripour et al. [61], in their two months study with mice reports a novel intravenous transplantation of very small embryonic like stem cells (VSELs). The transplanted cells were cultured with mouse embryonic fibroblast (MEF) cells and exhibited significant survival rates and proper hypoglycaemic activity in the pancreas (Figure 11). The VSELs are also reported to be contributing to the regeneration of pancreas [62].
In addition, Juliana Navarro Ueda Yaochite et al., 2015 [63] with their study with adipose-derived mesenchymal stem cells (ADMSCs) report a successful delivery and functioning of cells, via intrasplenic (I.Sp) and intrapancreatic (I.Pc) routes (Figure 12).

9. AGEING:

The transplantation of pancreatic β cells and their subsequent adaptation and functioning within the host tissue/vascular microenvironment, in regard to ageing is an important technical aspect to be considered. Studies on microcellular niche were conducted in muscle [64], skin [65] and neuronal cells [66]. Ageing is also associated with inflammation [67] and fibrosis [68] of pancreatic islets.
One of the interesting works is the heterochronic and isochronic transplant study by Seth. J. Salpeter et al. [69]. They transplanted β cells between young and aged mice. The proliferation rates were measured by the markers Insulin, Ki67 and Nkx6.1. The results indicated that β cells of both young and old mice proliferated significantly, when each of them were transplanted into young mice. In contrast, the cells exhibited attenuated proliferation rates when transplanted into old mice (Figure.13).

![Image of transplanted β cells](Adopted from Seth J. Salpeter et al., 2013)

**FIGURE 13:** Proliferation results of transplanted β cells with the markers Insulin, Nkx6.1 and Ki67 colored in green, maroon and blue, respectively.

### 10. CONCLUSION:

Various practically available interventions such as insulin, digestion regulators and insulin potentiators are relatively cheaper and could give a temporary remedy way before the acute failure of islets. All these factors, along with the costs of treatments make pancreatic islet replacement stand behind kidney, liver and heart transplantations. The reports on islet ageing strictly necessitate more research on cadaveric and xenotransplantation strategies and anticipate complementary gerontologic interventions. Attention must be given to the encapsulation and crosslinking materials, as they must remain inert in the long run and should not lead to any immune responses. Considerable evidences suggest [70-78] the association of pancreatic β cells with other networks, indicating certain ‘cross-talks’ among the heterodermal derivatives. These studies warn that the islets, albeit unique, are not isolated entities, but are in continuous equilibrium with other physiological systems. Any of our transplantation measures must be in harmony with these networks, to be optimally effective lest they elicit serious interdermal complications.
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