Exploring New Avenues in Islet Transplantation: Localized Immune Modulations with Rapamycin-Eluting Microparticles

by

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Abstract

Islet transplantation (ITx) is an effective means to restore physiologic glycemic regulation in those living with type 1 diabetes; however, lifelong systemic immunosuppression required to subvert the immune response remains a major barrier to patient inclusion. While these drug therapies delay the recurrent auto- and allo-immune response, adverse outcomes are often reported with their long-term usage. Chronic systemic immunosuppression can also give rise to opportunistic infections and malignant growths. Beyond other off-target toxicities, many of these drugs employed in ITx are diabetogenic which may explain the gradual decline in graft function seen clinically. Herein, we explored the use of a localized drug delivery system to preserve murine islet allograft function, circumventing the need for toxic systemic immunosuppression.

In this thesis, we encapsulated rapamycin (rapa), a potent immunosuppressive used in clinic ITx, into microparticles (MP) to achieve a localized release. Using a Food and Drug Administration-approved biodegradable polymer, poly(lactic-co-glycolic acid) (PLGA), enabled us to achieve a sustained drug release when co-transplanted with islets within mice. Once determining a non-toxic dose with an in vitro bioenergetic assay and an in vivo syngeneic ITx mouse model, we examined their ability to preserve allograft rejection in a fully major histocompatibility complex-mismatch ITx mouse model. When rapa-MP were co-transplanted with islets under the kidney capsule of diabetic recipients, we observed a significant prolongation in allograft function with 2/6 displaying long-term function for over 200 days. Non-drug-loaded MP controls all saw complete allograft rejection (4/4) by 19 days post-transplant. Combining the rapa-MP with a short course and low dose of cytotoxic T-lymphocyte-associated antigen 4 immunoglobulin (CTLA-4-Ig) yielded synergistic effects in preserving allograft function as all survived (6/6) long-term. These dual therapy recipients demonstrated a more robust response to a

glucose challenge at 100 d post-transplant when compared to naïve mice. To characterize the type of tolerance generated by rapa-MP + CTLA-4-Ig, we conducted a skin transplant study on mice with long-term functional allografts. An islet-graft or site-specific tolerance was concluded from these experiments as both donor-matched (to transplanted islet allografts) and third-party skin grafts were rejected. Next, we examined the cellular mechanisms that may confer the tolerance seen with intragraft gene expression analysis and saw that there was a downregulation of genes involved in adaptive immune pathways in rapa-MP co-transplanted grafts, which was potentiated further in the dual therapy grafts when being compared to empty MP control grafts. Lastly, we presented a proof-of-concept in examining rapa-MP in a humanized mouse model to test our technology in the context of a human immune system.

Overall, these results display the potential role of localized immunomodulation with drug-eluting MP to subvert the immune response in ITx. We display the role of rapa-MP in preserving long-term islet allograft function in mice, either as a monotherapy or when synergized as a combination therapy. Further testing the immunomodulatory potential of rapa-MP in a humanized mouse model may help us one day achieve an 'immunosuppressive-free' ITx approach in the clinical world.

Preface

This thesis presents original work performed by Jordan Wong, and collaborators mentioned in the Acknowledgment section. In part of the fulfillment of research responsibilities, Jordan Wong assisted with experiments and the work is presented in the published paper "Long-Term Survival and Induction of Operational Tolerance to Murine Islet Allografts by Co-Transplanting Cyclosporine A Microparticles and CTLA4-Ig" by P. Kuppan, J. Wong, S. Kelly, J. Lin, J. Worton, C. Castro, J Parmor, K. Seeberger, N. Cuesta-Gomez, C. Anderson, G. Korbutt, and A. Pepper (**Appendix A-2**). Jordan Wong assisted in developing an approach to analyzing donor human islets with the Extracellular Flux Analyzer XF24 which is reflected in a preprint article titled "HumanIslets: An integrated platform for human islet data access and analysis" by Ewald et. al (**Appendix A-3**). Learning opportunities in writing and collaboration with senior authors, Jordan Wong submitted work for publication "Pre-transplant Aerobic Exercise Improves Glycemic Outcomes After Marginal Islet Mass Transplantation in Rats" by J. Wong et. al and is awaiting a decision (**Appendix A-4**). Learning done in the CIRTN National Islet Biology course yielded a review article publication titled "Recent Developments in Islet Biology: A Review with Patient Perspectives" by L. Basu et. al.

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Glossary Terms

APC – Antigen-presenting cells

ASGPR – Asialoglycoproteinn receptor

AUC – Area under curve

BG – Blood glucose

BMI – Body mass index

CMRL – Connaught Medical Research Laboratories

CTLA-4-Ig – Cytotoxic T-lymphocyte-associated antigen 4 immunoglobulin

DCM – Dichloromethane

Dex – Dexamethasone

DKO – Double knock out

ELP – Elastin-like peptide

FDA – Food and Drug Administration

FKBP12 – FK506-binding protein 1A

HbA1c – Hemoglobin A1c

HBSS – Hank's Balanced Salt Solution

hESCs – Human embryonic stem cells

HLA – Human Leukocyte antigens

HOMA – homeostatic model assessment

HPLC – High-performance liquid chromatography

i.p. – Intraperitoneal

IBMIR – Instant blood-mediated inflammatory reaction

IEq – Islet equivalents

IPGTT – Intraperitoneal glucose tolerance test

iPSCs – Induced pluripotent stem cells

ITx – Islet transplantation

LBL – Layer-by-layer

MHC – Major histocompatibility complex

MMF – Mycophenolate mofetil

MP – Microparticles

MRI – Magnetic resonance imaging

mTOR – Mammalian target of rapamycin

mTORC1 – Mammalian target of rapamycin complex 1

mTORC2 – Mammalian target of rapamycin complex 2

NHP – Nonhuman primate

NICHE – Neovascular Implantable Cell Homing and Encapsulation

NOD – Non-obese diabetic

NAIDS – North American Islet Donor Score

NSG – NOD scid gamma

PBMC – Peripheral blood mononuclear cells

PBS – phosphate-buffer saline

PCL – Polycaprolactone

PEG – Poly(ethylene glycol)

PET – Positron emission tomography

PLG – Polylactide co-glycolide

PLGA – Poly(lactic-co-glycolic acid)

PVA – Poly(vinyl alcohol)

PVA – Polyvinyl alcohol

PVPON – Poly(N-vinylpyrrolidone)

Rapa – Rapamycin/sirolimus

RBC – Red blood cells

SEM – Standard error of the mean

SPECT – Single-photon emission computed tomography

STZ – Streptozotocin

T1DM – Type 1 diabetes mellitus

T2DM – Type 2 diabetes mellitus

TA – Tannic acid

TCR – T cell receptor

TGF-ß1 – Transforming growth factor-beta 1

Th1 – T helper 1 cell

Th2 – T helper 2 cell

TNF – Tumour necrosis factor

Treg – Regulatory T cells

Chapter 1: Overview of Islet Transplantation and Hurdles Ahead

1.1 Introduction of Diabetes

1.1.1 Brief Overview of Diabetes

The upward trend in diabetes mellitus has been a major worldwide health concern. With a 129.7% increase in the global prevalence of diabetes from 1990 to 2017, healthcare costs and disease morbidity are also on the rise[1]. As of 2017, approximately ~30% of Canadians are living with diabetes or prediabetes, and between 2011 to 2022 these cases are estimated to result in >$17 billion per year in associated healthcare costs[2]. These striking statistics reflect the significant societal burden of diabetes, necessitating the development of treatments and solutions that reduce disease morbidity. Further, it has warranted thorough investigations into the pathophysiology behind this metabolic disease.

Diabetes mellitus is characterized as a metabolic disease with the central symptom of chronic hyperglycemia. Hyperglycemia is often a result of either decreased insulin secretion from the pancreas, defects in the body's response to insulin, or a combination of the two[3]. As a result, chronic uncontrolled hyperglycemia can lead to long-term irreversible damage including both microvascular (retinopathy, nephropathy, and neuropathy, etc.) and macrovascular complications (coronary artery disease, cerebrovascular disease, peripheral vascular disease, etc.)[4]. These late-stage diabetes complications, in addition to the strenuous diabetes therapies, have a significant negative impact on patients' perceived quality-of-life[5]. People suffering from chronic hyperglycemia can be categorized into two broad categories: type 1 diabetes mellitus (T1DM) which is manifested by the autoimmune destruction of insulin-secreting cells (pancreatic beta-cells), and type 2 diabetes mellitus (T2DM) which is a consequence of beta-cell dysfunction and the development of insulin resistance, with the latter accounting for the majority

(~85%) of diabetes prevalence[6]. These two categories of diabetes have also been correlated with a decreased life expectancy as those living with T1DM and T2DM in the United Kingdom (UK) were estimated to have an average loss in life years of 7.6 and 1.7 years, respectively, compared to the general UK population[7]. Classic signs of diabetes include high fasting plasma glucose (above 7 mmol/L), decreased responsiveness to glucose (persistent hyperglycemia after controlled sugar consumption), elevated glycosylated hemoglobin A1c (HbA1c), and the presence of autoimmune markers (beta-cell autoantibodies), with the latter specifically pertaining to T1DM[8]. Diagnosing between T1DM and T2DM can be difficult, particularly among adults, as around 5-15% of patients are diagnosed with T2DM despite having autoantibodies present[9]. These findings may suggest that a significant portion of T1DM cases are misdiagnosed as T2DM.

Although the etiology behind T1DM is not fully elucidated, it has been established as a multifactorial disease resulting from the immune-mediated destruction of insulin-secreting pancreatic beta-cells within the Islets of Langerhans. As such, those with T1DM often require frequent exogenous insulin administration to maintain euglycemia. This mainstay treatment of multiple daily insulin injections has the inherent risk of potentially life-threatening hypoglycemia, for those with impaired awareness. On average, individuals with T1DM experience 1 episode of severe, disabling hypoglycemia per year, which can be accompanied by a seizure, coma, or death[10,11]. Preventative measures, such as the continuous glucose monitoring systems, allow those who inject insulin to monitor blood glucose levels more tightly throughout the day. In combination with insulin pumps, the use of a hybrid closed-looped system that continuously monitors blood glucose levels and automatically adjusts the delivery of rapid-acting insulin has been explored and was recently approved by the U.S. Food and Drug Administration

(FDA). Although this technology enables those living with T1DM to achieve improved glucose management, continuous accurate insulin infusion may fail from blockages or leakages[12]. Furthermore, the glucose monitoring sensors can become less accurate from anomalies including slow sensor signal attenuation, miscalibration, or dislodgment of the sensor from underneath the skin[12]. Hence, there remains a struggle to restore normoglycemia and improve glucose management in those living with T1DM without the typical and sometimes life-threatening complications associated with exogenous insulin therapy.

1.1.2 The Islets of Langerhans

The Islets of Langerhans are central to all forms of diabetes, therefore extensive investigations into their role in glucose homeostasis have been conducted. At least five distinct cell types have been identified in adult human islets: alpha-, beta-, delta-, pancreatic polypeptide, and ghrelin cells[13]. The main composition of human islets is alpha- (up to 65%)[14] and beta-cells (50-80%)[14,15], with dysfunction in the latter being central to the pathophysiology of diabetes. As such, understanding the cellular physiology of insulin secretion by beta-cells may prove advantageous. In brief, stimulation of the beta-cell with the uptake of circulating glucose leads to the metabolism and subsequent generation of ATP[16]. The elevated level of intracellular ATP triggers the closure of ATP-sensitive potassium channels, which are constitutionally open, resulting in a rise in membrane potential and depolarization. Voltage-gated calcium channels are activated and the sharp rise in Ca2+ facilitates exocytosis from reserves of insulin secretory granules. Insulin exocytosis is amplified and sustained beyond this trigger, though mechanisms are still under investigation. Consequently, insulin is released in an endocrine fashion, facilitating the lowering of blood glucose levels, and broadly speaking, triggering cellular glucose uptake and reducing gluconeogenesis and lipolysis[17]. Additional innervation of insulin release involves regulation via the autonomic nervous system, with the parasympathetic and sympathetic systems stimulating and inhibiting the secretion by beta-cells, respectively[18]. The complex influence of incretin hormones, peptides released

by enteroendocrine cells in response to glucose in the gut, have also been shown to alter insulin secretion and potentiate it in a glucose-dependent manner[19]. Ongoing work investigating the complex interplay between gut-brain incretin axis aims to uncover the intricate mechanisms involved[20,21].

1.2 History of Islet Cell Transplantation

Pancreatic islet transplantation (ITx) has become an established approach that frees recipients from severe hypoglycemic events, and insulin injections while improving glycosylated HbA1c. The modality of ITx has been explored as early as 1893, twenty-nine years before the discovery of insulin by Banting and Best. In December of 1893, Watson-Williams and Harsant attempted to treat a 13-year-old boy dying from ketoacidosis by performing the first documented islet tissue transplantation with pieces of sheep pancreas[22]. While minor improvement in glycosuria was observed, the boy rejected the xenograft and died comatose 3 days following transplantation[22]. Over twenty years later, Frederick Charles Pybus revisited ITx in a clinical study using subcutaneous implanted cadaveric human pancreas from recently diseased donors but also saw no success with patient survival[23]. In the 1950s, the hypothesis that the removal of exocrine acinar tissue was paramount in the viability and function of pancreatic grafts was well established[24]. With the islets of Langerhans making up a mere ~2% of the pancreas, islet isolation from adjacent exocrine tissue within the pancreas became a vital step for improving engraftment. This led researchers to perform laborious pancreatic microdissection to remove exocrine tissue under the microscope, resulting in poor yields and quality of islets; consequently, research efforts in the field of pancreatic fragment transplantation declined[25]. However, in 1965, Moskalewski introduced the method of collagenase-mediated isolation of guinea pig islets, revamping the field of ITx research[26]. In 1972, Ballinger and Lacy demonstrated the first-ever

experimental reversal of diabetes in rats through the transplantation of isolated islets within the peritoneal cavity and thigh muscles[27]. The following year, Kempt *et al.* demonstrated that isolated islets infused within the portal vein leading to the liver were the most effective and long-lasting site for the reversal of diabetes in rats, thus establishing a promising clinical site for ITx, one that is still used today[28] **(Figure 1.1)**. Two innovative surgeons in 1980, John Najarian and David Sutherland, explored this use of clinical intraportal ITx to preserve the endocrine function of 10 patients undergoing pancreatectomies for the treatment of chronic pancreatitis; following collagenase-mediated isolation of patients' own islets and successful infusion of these islets autografts into the portal vein, they found that four patients achieved insulin independence for at least 1, 9, 15, and 38 months, respectfully[29]. Although ITx with autografts would be ideal for decreasing the chances of alloimmune rejection, allograft transplantation (islets received from genetically non-identical donors) has been more frequently explored due to the scarcity of healthy islets in individuals living with T1DM. Despite these thrilling outcomes in ITx, the field failed to see large-scale success as the reproducibility of islet isolation was poor leading to low islet purity and yield. In this period, an automated and standardized islet isolation approach, the Ricordi Automated Method, was not widely employed[30]. Consequently, a mere ~8.2% of the 267 allograft transplant patients treated between 1980 to 1996 achieved insulin independence for greater than one year[31]. It was not until 2000 that Shapiro et al. pioneered a breakthrough, the *Edmonton protocol,* reigniting the flame of clinical ITx research[32]. The *Edmonton protocol* was the first ITx clinical trial to utilize newer and more potent immunosuppressive agents: sirolimus, tacrolimus and anti-CD25 antibody (daclizumab)[32]. The protocol also infused a larger number of healthy islets, isolated with the Ricordi Automated Method, into the portal vein compared to previous clinical studies (11,547 ± 1604 islet equivalents (IEq) per kg of recipient's body

weight)[32]. Shapiro et al. performed ITx on seven patients with T1DM, and their glucocorticoid-free immunosuppressive regimen demonstrated effective immunosuppression, circumventing the diabetogenic effects associated with glucocorticoid usage[32]. In fact, all seven T1DM patients achieved insulin independence for >1 year with functional insulin secretory function, indicated by sustained circulating C-peptide levels (a peptide co-secreted with insulin)[32]. The ground-breaking success of the *Edmonton protocol* sparked worldwide interest, inspiring >1000 ITx procedures in over 30 International transplant centers in the next two decades[33]. However, long-term follow-up of seven T1DM recipients enrolled in the multicenter international *Edmonton Protocol* failed to show sustained islet allograft function over a decade from their first infusion, with only one subject remaining insulin independent[34]. Evidently, the functional mass of islet allografts decreased over time as 20% of recipients remained insulin-independent at 10 years compared to 61% at 1 post-transplant in 255 transplants completed at a single-centre[35]. Where insulin independence may not always be achieved, ITx may still effectively reduce instances of severe hypoglycemic events while promoting euglycemia. A Phase 3 clinical trial by Hering et al. found that infusing islets into the portal vein of forty-eight patients with brittle T1DM led to 87.5% achieving an HbA1c <7.0 with no severe hypoglycemic events at 1-year post-transplant and 71% of patients sustaining these criteria by 2 years[36]. Despite the marked progress in the field of clinical ITx, the inability of this procedure to sustain long-term insulin independence warrants further developments in this field of cellular replacement therapy. Advancements may include further optimizing islet isolation and preparation, exploring alternative transplant sites that offer longevity in islet graft function, testing more effective and less toxic drug regimens, identifying alternative insulin-secreting cell sources (stem cell-derived and xenogeneic), and utilizing novel biomaterials and devices. Through these developments, cellular replacement

therapies may become a more practical, accessible, and promising ‘functional-cure’ for a broader range of people living with diabetes.

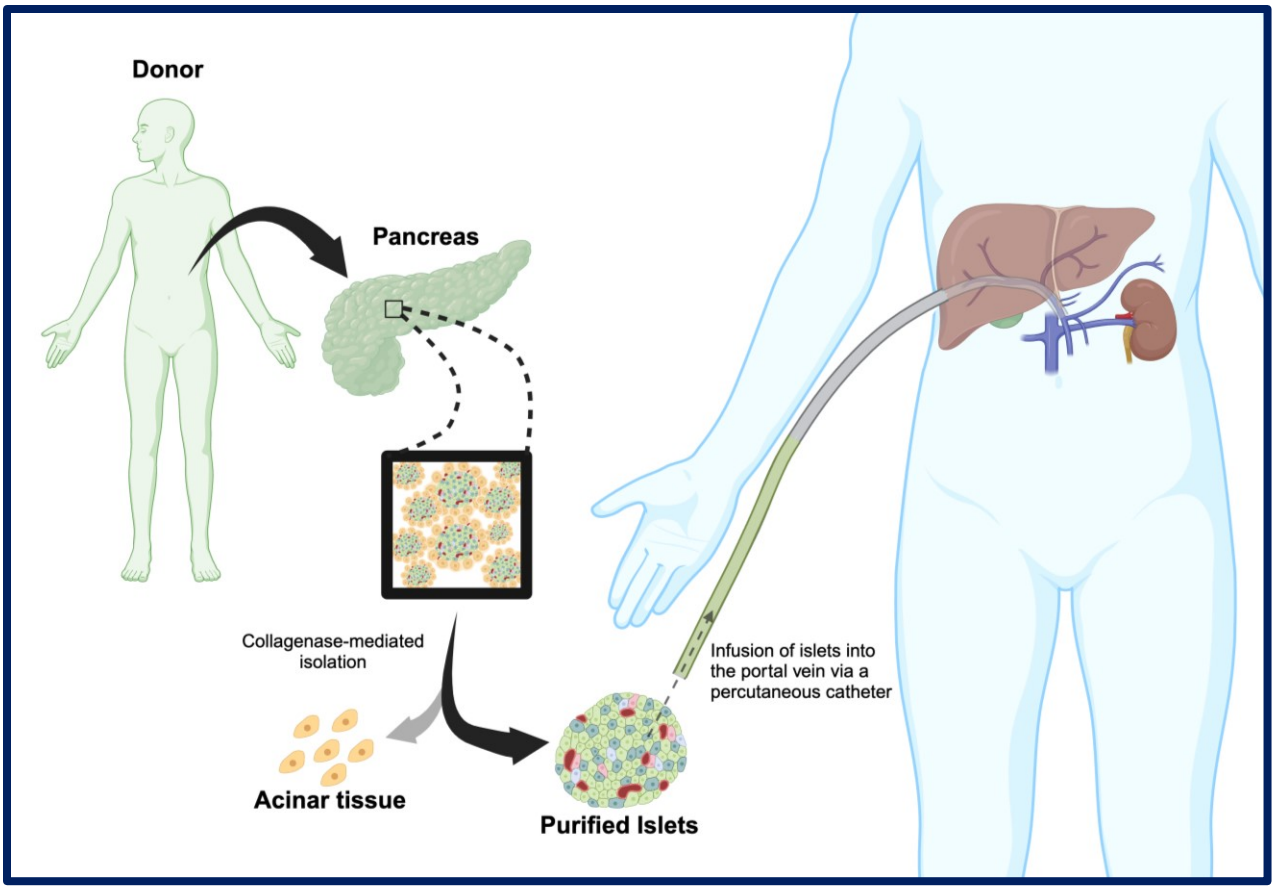


Figure 1.1: Broad methodology of clinical islet transplantation, involving collagenase-mediated islet isolation, percutaneous infusion of islets into the recipient's portal vein, and engraftment. Figure created by Jordan Wong with BioRender.

1.3 Current Status and Limitations of Islet Cell Transplantation

1.3.1 Donor Selection Criteria and Islet Availability

The limited number of viable islet donors has been a determining factor for the number of people afflicted with T1DM who can undergo ITx. With the human pancreas estimated to hold between 2.3-14.8 million islets[37], isolation outcomes aim to have high purity and yield (>300,000 IEq). Over the past two decades, multiple publications have attempted to correlate donor characteristics to the viability and outcome of islet isolation. Retrospective studies that examined donor body mass index (BMI), age, body weight, tissue cold ischemia time, hospitalization length, and HbA1c found that all these factors correlated with islet isolation outcomes[38-40]. Although these investigators strive to identify ideal donor characteristics with these studies, interpretation of results must be done with caution. A large portion of retrospective analysis identifies ideal donor characteristics based on high isolation yields[38-40], but a large number of islets is not always a good indicator of islet function. Herein lies room for error in translating findings to optimal islet physiology and graft performance. This is well illustrated by a study reviewing 153 human islet isolations, in which older donors (age 51-65) produced a significantly higher islet yield (>100,000 IEq) and purity compared to younger donors (age 2.5-18)[41]. However, they also measured the insulin stimulation index, an indicator of islet function, and found that younger donor islets demonstrated significantly superior insulin secretory capabilities compared to the group of older donors[41]. Therefore, the current method of defining ideal donors in the literature may be misrepresentative of the true definition of an 'ideal' donor in terms of optimal islet physiology. Ongoing examinations, led by the University of Alberta and Alberta Diabetes Institute's HumanIslet Core, into the phenotype, bioenergetics, metabolism, and omics of isolated islets and comparing these outputs to donor characteristics may provide new insights

into what constitutes an 'ideal' donor **(Appendix A-3)**. Nevertheless, standardizing donor selection for ITx may help to improve long-term success.

The first scoring system based on donor characteristics was developed by O'Gorman et al. and has been used to determine whether a pancreas would be viable for clinical islet isolation[42]. The system was developed at a single isolation centre based on 326 donors between 1999 and 2004[42]. More recently, Wang et al. developed the North American Islet Donor Score (NAIDS) to assess pancreas selection for appropriate clinical transplantation with increased accuracy[43]. Similar to O'Gorman's system, the NAIDS acts as a diagnostic tool for clinical decision-making based on donor characteristics, however, this system was developed through retrospective multicentre analysis of a larger data set: 1,056 donors across 11 islet isolation centres in North America[43]. Moreover, the NAIDS has been validated and remains to be the most useful and available tool for donor pancreas selection to date[44]. Future advancements in scoring systems may benefit from developing criteria based additionally on the relationships between graft performance, length of insulin independence, and islet insulin secretory ability to donor characteristics. Efforts to elucidate this relationship with ITx outcomes may improve the discriminative abilities of the scoring system, allowing clinicians to identify donors that would likely have favourable outcomes in both islet isolation and transplantation.

Akin to other organ transplantation, there is a growing disparity between the availability of donors and the climbing numbers of eligible patients that can benefit from ITx. In 2019, it was estimated that 463 million people worldwide were living with diabetes and its associated complications[45]. That same year, the Global Observatory on Donations and Transplantation registered 40, 608 deceased organ donors[46]. Assuming that ~10% of the estimated global diabetes population suffers from T1DM, and that all organ donors were eligible for islet isolation

and transplantation, only 0.088% of the global T1DM population could receive a single donor transplant in 2019; patients routinely require multiple islet donors to achieve insulin independence. Furthermore, not all organ donors would fit the eligibility criteria for islet isolation and transplantation. These considerations may suggest that an even lower percentage of those living with T1DM can undergo ITx, widening the disparity between islet supply and treatment demand. Therefore, clinical ITx has been limited to those living with brittle diabetes and life-threatening hypoglycemic unawareness[32]. As such, there is a drive to identify a less limited, alternative insulin-producing cell source.

1.3.1.1 Alternative Cell Sources: Xenogeneic Islets

The use of islets that originate from different species has been widely explored in order to circumvent the demand for human donors. Multiple sources of xenogeneic islets have been investigated including tissue derived from bovine[47], fish[48], sheep[49] and porcine (pig)[50]. Although each source has associated advantages and disadvantages, at present, pig islets prove to be the most promising source due to their similar physiological and morphological features to human islets. Additionally, pigs are an attractive source because of their i) high fecundity, ii) efficiency of genetic modification through well-established techniques, iii) practicality of housing them under pathogen-free conditions, and iv) cost-efficient feasibility[51]. Furthermore, porcine insulin has been used as an established and effective diabetes therapy for over 2 decades, demonstrating that pig islets may serve as a promising alternative cell source for ITx[52]. Multiple preclinical studies support this notion; xenotransplantation of neonatal porcine islets has been demonstrated to be an effective means of achieving long-term reversal of diabetes in diabetic rodents[53] and nonhuman primate (NHP) models[54,55] in adjunct to immunosuppressant treatment. However, translating these experimental successes to a clinical setting remains a major challenge. The first

ever human islet xenotransplantation of 10 patients with fetal porcine islets was performed by Growth et al. in 1994[56]. They demonstrated that xenografts can survive for up to serval months, but patients failed to show any improvements in their glycemic control[56]. Since then, investigators have made procedural adjustments including genetic modifications, islet encapsulation, and isolation modifications in an attempt to improve the clinical success of xenotransplantation. In 2014, Matsumoto et al. transplanted 14 patients with unstable T1DM with encapsulated neonatal porcine islets without immunosuppression and saw a reduction in unaware hypoglycemic events, but only a minimal reduction in HbA1c and daily insulin dosages at 1-year post-transplant[57]. A similar 2016 clinical study in Argentina, where 8 patients underwent intraperitoneal transplantation with encapsulated neonatal porcine islets, also saw patients experiencing fewer episodes of unaware hypoglycemia and an improvement in HbA1c, but no change in daily insulin injections[58]. Beyond ITx, recent thrilling advancements in clinical xenotransplantation highlighted the genetic modification approach. Two separate patients were transplanted with a genetically modified pig heart or kidney with 10 and 69 gene edits, respectively[59,60]. Triumphantly, no acute rejection was seen with both procedures with the pig heart failing at 48 days and the pig kidney at just under two months after transplantation. These thrilling cases shed light on the possibility of a functional alternative organ source that may help fill the demand for organ transplantation. Exploring the clinical transplantation of islets from these genetically modified pigs may address the immunological barriers of xenotransplantation. Certainly, more work is necessary in the field to provide long-term success, and if successful, could represent an unlimited source of organs.

1.3.1.2 Alternative Cell Sources: Stem Cells

Another alternative cell source that has been heavily investigated for ITx is stem cells. The attractive benefits of stem cell therapy include the unlimited cell source and their suitability for immune tolerance. There has been increasing interest in functionalizing and generating insulin-secreting cells from human embryonic stem cells (hESCs) and induced pluripotent stem cells (iPSCs). To achieve such a feat, extensive steps are required to differentiate these stem cells into glucose-responsive and insulin-producing cells. In 2001, Assady et al. demonstrated that hESCs could spontaneously differentiate into an array of cell types, including those that produce insulin[61]. Following this discovery, Segev et al. modified a differentiation protocol initially used to generate insulin-producing cells from mouse ESCs and were able to successfully differentiate hESCs to secrete a substantial amount of insulin[62]. Further exploration of hESC differentiation protocols led to the successful production of more mature, glucose-responsive, and insulin-expressing endocrine cells[63]. In 2008, this protocol was used to generate hESC-derived pancreatic endoderm that was transplanted into diabetic-induced mice, effectively reversing hyperglycemia and indicating the potential for clinical usage[64]. Translation to the clinical setting has been promising, as three clinical trials that implanted pancreatic endoderm cells showed effective insulin expression and C-peptide secretion at 1-year post-transplant[65-67]. The alternative stem cell source, iPSC, is a promising approach due to the possibility of an autologous transplantation. The protocol to dedifferentiate human skin fibroblast to human pluripotential stem cells was first discovered by Yamanaka's group in 2006[68]. Following dedifferentiation mediated through the Yamanaka genetic factors (Oct3/4, Sox2, c-Myc, and Kl4)[68], many demonstrate the ability of iPSCs to differentiate into insulin-producing beta-cells[69,70]. Even so, their ability to form mature pancreatic endocrine cells remains inferior to products from hESC protocols[71], proving an area for further developments in iPSC

differentiation protocols. Potential for iPSC is apparent as autologous stem-cell derived islet infusion into the portal vein of a T2DM patient effectively reduced their exogenous insulin requirement, all without immunosuppression[72]. Furthermore, ITx of allogeneic stem cell-derived islets restored insulin independence in two T1DM patients and ongoing transplants are being conducted[73]. Although intense lifelong suppression may not be required with autologous iPSC-based transplantation as there is an absence of allorecognition, the possibility of recurrent autoimmunity is an area of concern. Furthermore, hESC and iPSC differentiation strategies require major monetary and time investments, which may bring into question their feasibility. Regardless, both avenues of hESCs and iPSCs may one day serve as an alternative cell source for ITx.

1.3.2 Assessing Islet Graft Function

The significant improvements in experimental ITx research along with clinical advancements demonstrate the ability of researchers to assess allograft function and adjust protocols accordingly. Having an accurate monitoring system provides investigators with more information on the impacts of treatment. Similar to standardizing ITx protocols and donor selection, creating a scoring system to monitor islets objectively has been a major area of interest. To achieve this, monitoring graft function is a key measure of ITx outcomes, which can help direct new alterations and improvements to current treatment protocols. Furthermore, using multiple indicators to assess graft function can help create standardized and validated scoring systems that eliminate some biases with clinical observation.

Currently, the main factors used as objective measures of graft function are clinical indicators. Measures in controlled glucose tolerance tests, fasted circulating C-peptide, HbA1c, daily exogenous insulin requirements, and renal function have been evaluated individually or in

combinations to create standardized scoring systems. To name a few, these systems include the homeostatic model assessment (HOMA) -beta score[74], a secretory unit of islet transplant objects[75], transplant estimated function[76], and most recently the BETA-2 score[77]. Providing quantitative and objective measures with these scoring systems allows clinicians to further adapt treatment to improve the success of ITx.

Visualizing islet grafts serves as complementary information to the clinical parameters discussed above. Imaging systems evaluated experimentally like positron emission tomography (PET), single-photon emission computed tomography (SPECT), magnetic resonance imaging (MRI), and ultrasound imaging paint a larger picture by visualizing biological processes at a cellular level[78]. At present, there is a lack of a standard, clinically relevant, and non-invasive imaging method to monitor islet grafts in the intrahepatic site, however, there are ongoing efforts to validate such an imaging technique.

1.3.3 Limitations of Clinical Portal Vein Infusion

As of today, virtually all clinical ITx worldwide are mediated through intrahepatic islet infusion. While this method of percutaneous intraportal pancreatic islet infusion is a minimally invasive procedure and an effective means to achieve insulin independence, it is not without expected risks including portal vein thrombosis, hepatic steatosis, and intraperitoneal bleeding from hepatic punctures[79]. Furthermore, despite the refinements made in islet isolation and transplantation protocols over the last two decades, intrahepatic islet transplantation is still associated with an immediate loss of 50-70% post-transplantation[80,81]. This acute islet cell death in the peri-transplant period compromises the long-term success of reversing diabetes and severely limits engraftment. Furthermore, a larger number of islets is required per recipient, further restricting the number of T1DM patients that can be treated with the already limited

donor supply. Factors that may contribute to early graft loss specific to the portal vein microenvironment include the instant blood-mediated inflammatory reaction (IBMIR), activated endogenous liver immune cells, and islet hypoxia. Understanding and exploring these barriers may help identify clinical interventions that could improve early graft survival and alternative solutions/sites that are more favourable than the liver microenvironment.

The instant blood-mediated inflammatory reaction (IBMIR) is a well-studied and early consequence of intrahepatic islet infusion. This complex and nonspecific innate immune response is a major cause of the acute destruction of islets post-transplantation[82]. The IBMIR results in a coagulation cascade that is triggered by two islet-specific factors that promote platelet binding: the negatively charged islet surface[83] and the external expression of tissue factor on the islets[84]. Since the graft is infused directly into the bloodstream via the portal vein, there is ample opportunity for circulating platelets to interact with these coagulation triggers on the islet surface. Following the formation of macroscopic clots, a panel of cytokines are released, and inflammatory cells are recruited and activated[85]. There have been multiple attempts to protect islets against hypoxic and inflammatory stress associated with IBMIR. In experimental models, the coating of islets with endothelial cells[86] and the infusion of anti-coagulates including heparin[87], low-molecular-weight dextran sulfate[88], and thrombin inhibitors[89] have all shown to be effective means of disrupting the IBMIR response. However, to date, only heparin has been validated in clinical settings[90].

As previously mentioned, IBMIR involves the release of cytokines that promote the recruitment and activation of inflammatory and immune cells. The liver has specific endogenous immune cell types, however, there is limited data exploring the impact of these cell types on intrahepatic infused islets. Nevertheless, the endogenous liver immune system may also be

considered as a potential drawback and inflammatory propagator of the hepatic site. These cell types include liver-specific phagocytes (Kupffer cells), liver sinusoidal endothelial cells, hepatic stellate cells, resident liver lymphocytes and dendritic cells, and hepatocytes[91]. Studies identifying the roles that each of these cell types plays in immediate and gradual graft attrition may help fine-tune the immunomodulatory regimen prescribed to transplant recipients.

Native islets within the pancreas are well-oxygenated, as they make up only 1-2% of the pancreas' total volume but receive 10-15% of its blood flow[92]. However, through the isolation process and in culture conditions, they suffer from a drop in oxygen delivery and consequently undergo cell death[93]. Unlike full organ transplantation, islet grafts are simply infused into the portal vein and are not anastomosed to blood vessels, thus experience reduced oxygen availability and hypoxia exposure until angiogenesis forms a functional circulatory system within a 10–14-day period following transplantation[94,95]. Hence, islets are mainly oxygenated through diffusion in the early stages of engraftment, which is further impaired by the low oxygen tension of the portal vein system. Moreover, these islets have been shown to experience a persistent and chronically low drop in endogenous oxygen tension, going from an initial 40 mmHG within the pancreas to a meager 5 mmHG in the portal vein for up to 3 months post-transplant[92,96]. Consequently, the hypoxic environment is a trigger for cell death, thus resulting in a major loss in islet graft mass. The evident hypoxia among other hepatic factors contributes to poor engraftment, and therefore a larger number of islets is required at this site. In addition, the intrahepatic site still poses a challenge for graft imaging and retrieval, which can be more detrimental in removing malignancies if alternative cell sources such as stem cells are used. As such, these factors may suggest the liver is not an optimal site for islet transplantation.

1.3.4 Transplant Site Independent Factors Contributing to Loss in Functional Islet Mass

Though there has been major progress in clinical islet isolation, this extensive 5–7-hour multi-step process remains detrimental to functional islet mass. As explored earlier, islet isolation consists of cold enzymatic digestion, which is later followed by mechanical shearing, density gradient purification, and cell culturing. The early development of the Automatic Method utilizing the Ricordi Chamber and continuous digestion-filtration pancreas processing eliminates some human error and has been shown to substantially improve the qualitative and quantitative clinical isolation outcomes[30,97]. Over the next three decades, the Automated Method has evolved to further improve islet isolation outcomes and remains the central technology in clinical islet processing facilities worldwide[24]. Despite these advancements, this process can be extremely stressful to sensitive beta-cells, therefore leading to loss in functional islet mass. The current isolation and purification procedures destroy the islet capillary network, thereby preventing the delivery of adequate oxygenation to the level of a normally functioning pancreas. As such, islets experience a period of acute hypoxia throughout isolation, which has been shown to induce apoptosis in beta-cells through upregulating the pro-apoptotic transcription factor C/EBP homologous protein (CHOP) in vitro[98]. Moreover, evaluation of human islets immediately following isolation revealed that ~30% of all islets stained positive for apoptosis (terminal dUTP nick end labelling (TUNEL) staining), with beta-cells representing the largest proportion of stained cells[99]. Human islets transplanted into immunodeficient nude mice also demonstrated a large loss in functional islet mass as they measured up to a 70% decrease in beta-cell mass by 1-month post-transplant[100]. Other murine studies demonstrate a similar trend, with this profound reduction in islet mass being independent of the transplantation site[82,101].

Another major contributor to graft attrition is the immune response. Recruitment of macrophages following the inflammatory reaction, which is propagated by the IBMIR, results in migration and activation of cytotoxic T cells (CD8+) that directly contribute to islet cell death. These mechanisms will be explored further in the next section; however, it is vital that the recipient's immune reaction is identified as a contributor to the loss of functional graft mass. For this reason, transplant recipients are required to undergo long-term immunosuppression treatment for the length of the graft function in order to prevent rejection. Despite these efforts to prolong graft function, chronic immunosuppression has potentially serious side effects including the risks of developing debilitating infections or malignancies. Furthermore, immunosuppressants used in the past have also been shown to have diabetogenic properties as a result of direct harmful effects on beta-cell function[102-104]. Subverting the immune response while minimizing immunosuppressant toxicities remains a major challenge in ITx, though the development of more selective and potent drugs over the years has been majorly beneficial.

1.4 Immunomodulation: A Fine Line

1.4.1 Overview of the Auto- and Alloimmune Response

The majority of patients undergoing ITx are afflicted with T1DM, and as such, are susceptible to two distinct types of immune-mediated graft destruction: the alloimmune response to the foreign islets, and the recurrent autoimmune response that is the main driver of the initial onset of this metabolic disease. Multiple studies have worked on identifying pathways and immune cells involved, along with determining the roles each contributes to ITx outcomes. Despite the ongoing investigation, there is still an unclear understanding of which process is the main instigator of immune rejection. Nevertheless, distinguishing the mechanisms of these two

types of immunity can help generate novel targeted treatments and transplant approaches that could decrease the occurrence of graft rejection.

As many transplant recipients are within the later stages of T1DM, indicated by a large portion of immune cell-mediated beta-cell loss, there is a high probability of recurrent autoimmunity. Before diving into the mechanism of recurrent autoimmunity, it may be useful to further explore T1DM pathogenesis. In 1974, the immune system was first suggested to play a role in T1DM when Nerup et al. discovered an association between the type of human leukocyte antigens (HLA) complex and insulin-dependent diabetes[105]. The HLA system, also known as the major histocompatibility complex (MHC), is primarily involved in antigen presentation to elicit a targeted immune response. Specifically, HLA class I (MHC class I) molecules are ubiquitously expressed on the plasma membrane of almost all nucleated cells and present cytosolic peptides to CD8+ T cells through interaction with T cell receptors (TCRs), while HLA class II (MHC class II) is exclusively expressed on B lymphocytes, antigen-presenting cells (APCs), and activated T lymphocytes to detect circulating antigens and present them to CD4+ T cells also via TCRs[106]. A heightened sensitivity in MHC results in immune reactions to a larger panel of antigens. As such, the HLA system is responsible for foreign antigen detection, and in cases of autoimmune diseases, endogenous antigens. Since the initial association between HLAs and T1DM, genome-wide association studies corroborate the significance of antigen presentation, as they found that up to 50% of HLA genes (notably HLA class II genes) accounted for the genetic risk of T1DM[107,108]. Furthermore, a study controlling for HLA class II alleles found that HLA class I genes are also associated with T1DM[109]. Nonetheless, there remains a lack of consensus on an identified primary autoantigen involved in T1D, possibly due to the heterogeneity of this metabolic disease. Islet-specific autoantigens that have been considered include proinsulin

(precursor of insulin), zin transporter 8 protein (essential for biosynthesis and secretion of insulin), insulin promoter factor 1 (IPF-1), islet amyloid polypeptides (peptide hormone co-secreted with insulin), etc.[110]. There is a general hypothesis that beta-cell autoantigens are processed through the APCs HLA class II molecule complex, resulting in activation of naïve T cells to autoreactive CD4+ T cells. Following activation, these autoreactive T cells migrate to the pancreas and locally release a panel of cytokines to stimulate macrophage and T cell-mediated beta-cell destruction[111]. These views are supported by the following findings: i) the presence of infiltrated T cells in T1DM patient inflamed islets (insulitis) at the onset of T1DM[112]; ii) T cells obtained from within islets of T1DM donors were highly reactive to an autoantigen (preproinsulin)[113]; and iii) systemic immunomodulators targeting T cells delayed disease progression in clinical studies[114]. Although the cytotoxic mechanisms driving T1DM remain elusive, there is strong evidence that HLA complex-mediated antigen presentation and T cells are involved in autoimmunity.

Clinical and preclinical experimental outcomes of ITx have been associated with a recurrent autoimmune response. Similar immune events responsible for the onset of T1DM have also been seen in the period following ITx. In non-obese diabetic (NOD) mice, an autoimmune model of T1DM, MHC class II mismatching between donors and recipients demonstrated longer graft survival compared to when the donor and recipient shared similar MHC class II antigens[115]. Moreover, studies utilizing a rat model of autoimmune T1DM also found that performing ITx with MHC-mismatched grafts demonstrated prolonged survival and were not susceptible to recurrent autoimmunity compared to MHC-matched grafts[116,117]. Altogether, these preclinical findings provide further evidence of the significant role of MHC antigen presentation in T1DM autoimmunity. In the clinical setting, characterizing recipients' immune cell reactivity against

islet autoantigens pre- and post-transplant can help elucidate the relationship between recurrent autoimmunity and transplant outcomes. A study exploring this relationship performed multivariate analyses of 21 people living with T1DM and demonstrated that the presence of cellular autoimmunity immediately prior to and one year following ITx was associated with significant delays in achieving insulin-independence and inferior graft function, indicated by lower circulating C-peptide levels[118]. Furthermore, a study linking clinical ITx to increased rates of post-transplant autoreactive memory T cell proliferation provides greater evidence for recurrent autoimmunity in patients with T1DM[119]. Although the mechanisms underlying the recurrent autoimmune response seen in T1DM patients remain unclear, clinical and preclinical findings provide evidence for its occurrence and possible contributions to long-term graft failure.

The alloimmune response is another major concern that contributes to unfavourable ITx outcomes. This process is driven by alloreactive T cells that respond to the genetic dissimilarities between the recipients and the islet graft tissue. Similar to autoimmunity, the MHC complex plays a vital role in eliciting an immune response. Specifically, host immune cells are directed against unfamiliar MHC class I molecules that also ubiquitously present foreign peptides found within the allograft cells, the foreign nature of donor MHC I molecules themselves, and MHC class II on recipient's APCs that uptake circulating foreign antigens originating from the graft[106,120]. CD8+ T cell activation through foreign antigens presentation on allografts, mediated by foreign MHC class I recognition, leads to CD8+ cytotoxic T cell-mediated islet destruction and the triggered release of cytokine that precipitates inflammation and coagulation[120]. Consequently, blood flow to the islets becomes disrupted and graft ischemic injury occurs. As mentioned, hypoxic conditions have been linked to cell death and upregulated genes in the cell death pathway which contribute to graft failure and metabolic dysfunction[98,99]. Additional

activators of the alloimmune response involve the MHC class II molecules on APCs that present foreign antigens to activate CD4+ helper T cells[106]. Subsequently, these CD4+ helper T cells activate macrophage through cytokine release along with promoting the production of antibodies via B lymphocyte activation[120]. A study in which investigators reconstituted immunodeficient mice with a mix of lymphocytes that excluded alloreactive T cells demonstrated long-term islet allograft survival using mismatched MHC complex between donor and recipients[121]. The alloimmune reaction generated through the mismatch between donor and recipient MHC molecules is a major contributor to allograft rejection.

1.4.2 Overview of Immunomodulatory Agents Used in Clinical Islet Transplantation

The struggle to suppress immune rejection has been an ongoing battle since the first initial islet mass transplant in 1893. Utilizing immunosuppressive agents has allowed the field to achieve and prolong insulin independence; however, there has yet to be any long-term success in curing T1DM. Nevertheless, immunosuppressants have been a major contributor to allogeneic ITx success and have evolved throughout the years. The goal of immunotherapy is for recipients to develop a tolerance phenotype to transplanted donor tissue, thus preserving graft function and survival. In the clinical setting, there are two phases of immunosuppressant treatment to achieve this tolerance: induction and maintenance immunosuppression.

Induction agents target immunity that would be heightened during transplantation to reduce the incidence of acute rejection. Sustained induction therapy would most likely result in iatrogenic events, therefore prolonged use is not ideal. Practically all transplantation employs induction therapy, including ITx, but there is no global standard ITx immune modulation regimen. Typically, induction therapy is employed ~1-2 days prior to transplantation and is administered up to 7 to 14 days post-ITx. Induction therapy that has T cell-depleting actions has

been shown to have a positive effect on long-term insulin independence, regardless of the type of maintenance immunotherapy later employed[122]. Thus induction therapy has been and remains a crucial step in clinical ITx[122]. The following induction agents are typically used in clinical ITx and the corresponding mechanism of action (**Table 1.1).**

Following transplantation, higher concentrations of immunosuppressants are initially used to prevent acute rejection and over time, a lower dose is prescribed to decrease the risk of toxicities associated with chronic treatment. This maintenance immunomodulatory regimen aims to protect islet grafts from the allo- and recurrent auto-immune responses that were previously explored. Since this lifelong immunosuppressive therapy is a crucial treatment for transplant recipients, immunosuppressants over the years have constantly evolved to prolong islet function, while still having minimal toxicity. This can be achieved with potent and selective agents with minimal off-target effects. The following agents have been commonly used as maintenance immunosuppressive therapy in ITx, and the associated mechanisms (**Table 1.2).**

Table 1.1: Mechanism and uses of immunosuppressives used in the induction phase of ITx.

Glucocorticoids	Prednisone is a synthetic, anti-inflammatory glucocorticoid that supresses the immune system through altering gene expression. Through binding nuclear receptors, prednisone inhibits the production of proinflammatory cytokines resulting in decreased circulating lymphocytes[110]. Prolonged use of high-dose glucocorticoids has been seen to cause serious adverse effects to systems including musculoskeletal, cardiovascular, gastrointestinal tract, and the endocrine system[110]. Glucocorticoids have also been seen be diabetogenic which further serves as an additional barrier in ITx[110]. Therefore, acute use of these agents in the induction phase of ITx have been explore and are currently still used in the clinical setting[123-125].
Daclizumab (Dac)	Used in the *Edmonton Protocol*[126], Dac is a humanized and monoclonal antibody that inhibits interleukin-2 receptor (IL-2R) via reversible CD25 blockage, the high-affinity subunit on the IL-2R. IL-2 is the main ligand that activates the IL-2R and is released by activated T cells. IL-2 receptors are expressed on a number of immune cells, notably activated T cell, memory CD8+ T cells, naïve T cells, and natural killer T cells[127]. Additionally, regulatory T cells (Treg) express IL-2R, and signaling within all these cells promote proliferation, and for some lymphocytes (CD8+ T cells, effector T cells, etc.) are essential for differentiation and activation[127]. Dac blockage of IL-2R signaling thus inhibits induction of the immune response, preventing acute immune rejection.
Basiliximab	Targets identical pathways as Dac, but is a chimeric monoclonal antibody produced through recombinant technology[128]. Basiliximab blocks the same IL-2R subunit as Dac, and thus supresses immune cell proliferation and maturation. Both Dac and basiliximab are commonly used in renal transplantation to prevent occurrences of acute rejection, and a meta-analysis of 6 randomized controlled trials (total of 509 patients) demonstrated that basiliximab and Dac had similar efficacy and safety profiles[129]. Therefore, clinicians have used basiliximab and Dac interchangeably as induction therapy in ITx.
Anti-thymocyte globulin (ATG, Thymoglobulin)	A polyclonal, rabbit anti-thymocyte globulin that rapidly depletes T cells and other lymphocytes. The main mechanism of lymphocyte depletion is complement-dependent cell lysis[130]. Consequently, there are less active T cells that can precipitate an allo- and auto-immune reaction in the peri-transplant period. Thymoglobulin has been utilized in a clinical setting for over 30 years[131], and is currently still used as an immunosuppressant in solid-organ transplantation[132].
Tumor necrosis factor (TNF) inhibitor (Etanercept)	Etanercept works through biologically inhibiting pathways involved in the development and progression of inflammation, TNF receptor 1 (TNFR1) and TNF receptor 2 (TNFR2). TNFR1 agonism via the endogenous ligand (TNF-α) binding triggers a proinflammatory response, while activation of TNF2R on immune cells, also by TNF-α, promotes immune cell survival and proliferation[133]. Thus, inhibition with etanercept supresses the inflammatory response and immune cell proliferation following

	transplantation[133]. These effects also extend to Treg cells, suppressors of activated immune cells, therefore Etanercept is used with caution in ITx. Moreover, high concentrations of this TNF inhibitor has been revealed to reduce islet function and integrity[134]. Etanercept is often used in combination with ATG or alemtuzumab as an ITx induction therapy.
Alemtuzumab	Alemtuzumab is a monoclonal and humanized antibody against CD52 found on the membrane glycoprotein of T and B lymphocytes, NK cells, macrophages, and other immune cells. The function of CD52 is still unclear, however some have suggested that this pathway may be involved in T cell co-stimulation and migration[135] along with Treg induction[136]. Therefore, alemtuzumab administration has been seen to cause significant T and B lymphocyte depletion.
Anakinra	Another anti-inflammatory agent typically used with etanercept in the induction phase. Anakinra competitively binds IL-1R, thereby inhibiting the proinflammatory actions of IL-1[137]. Agonism of IL-1R, stimulated via damage recognition (during transplantation), triggers the production of a cascade of inflammatory cytokines including TNF-α[137]. Therefore, inhibiting this signaling pathway would be ideal for decreasing the inflammatory and immune recruitment response immediately following ITx.

Table 1.2: Mechanism and uses of immunosuppressives used in the maintenance phase of ITx.

Cyclosporin (Cyclosporine A, CsA)	Early biological studies demonstrated potent immunosuppressive abilities of CsA through blocking the transcription of cytokine genes (IL-2 and IL-4) that are necessary for T cell activation[138,139]. It was later discovered that these effects were mediated through inhibition of the calcium and calmodulin dependent serine/threonine phosphatase, calcineurin. Calcineurin is stimulated via calcium and calmodulin during T cell activation, where it dephosphorylates nuclear factors of activated T cells (NFAT), which activates these proteins and allows them to translocate to the nucleus[140]. Once NFAT are in the nucleus, they bind DNA and associate with other transcription factors to promote transcription of cytokines: IL-2, IL-4, IL-10, and IL-17[141]. CsA mediated calcineurin inhibition prevents NFAT dephosphorylation, decreasing the transcription of cytokines that are vital propagators of the allo- and auto-genic immune response. Specifically, this blockage is achieved through CsA binding to the immunophilin, cyclophilin A (predominantly found in T cells) and this complex has enhanced selective affinity for calcineurin, thus inhibiting its phosphatase ability[141]. The effect on IL-2 has been thought to be the main contributor of immunosuppression. As explored earlier, IL-2 is necessary for the action, survival, and differentiation of CD4+ and CD8+ T cells[127].
Tacrolimus (FK506, tac)	A macrolide antibiotic that supresses the immune system in a similar way to CsA. However, tac binds to a different immunophilin, the FK506 binding protein (FKBP), which also leads to the inhibition of calcineurin[142]. As such, tac and FKBP complex-mediated calcineurin inhibition results in decrease cytokine gene transcription, therefore decreasing T cell proliferation. One main difference between CsA and tac is that the latter is around 100 times more potent, which may be a reason for tac gaining popularity over the years for easier dosage[142]. Moreover, tac has the capacity to reverse phases of allograft rejection when the use of steroids becomes ineffective[143]. Thus, tac is an ideal agent for maintenance immunosuppression, and was used in combination with sirolimus in the *Edmonton protocol*[32].
Sirolimus (rapamycin)	Veniza and colleagues discovered rapamycin (sirolimus) on Easter Island in the early 1970s and identified it as a product of *Streptomyces hygroscopicus*[144]. Although rapamycin was initially isolated as an antifungal agent, later studies revealed potent immunosuppressive activities through inhibiting the mammalian target of rapamycin (mTOR), a vital component in immune cell maturation, function, and proliferation. mTOR is a serine-threonine kinase that functions through two distinct complexes: mammalian target of rapamycin complex 1 (mTORC1) and mammalian target of rapamycin complex 2 (mTORC2)[145]. Rapamycin is proposed to interact with a binding protein (immunophilin FK506-binding protein 1A,

	12kDA (FKBP12)) to form a complex that specifically blocks mTORC1[146]. The FKBP12-rapamycin complex binds the amino-terminal of mTORC1, disrupting cell growth by reducing translation, ribosome biogenesis, and autophagy. Moreover, mTORC1 plays a major role in regulating cell growth and downstream processes in immune cell development, thus FKBP12-rapamycin mTORC1 blockade impairs dendritic cell maturation and function, and inhibits T cell and B-cell proliferation[146]. In rodents, rapamycin mediated mTOR blockage caused significant thymus atrophy, associated with lower T cell output[147]. Moreover, in vitro exposure of rodent and human CD4+ CD25+ Treg cells to rapamycin did not impair Treg-dependent immune suppression, and conversely promoted expansion of functional Tregs cells in T1DM patients[148]. These potent immunosuppressive effects contribute to the crucial tolerance phenotype necessary in islet engraftment, and is the reason that rapamycin remains one of the most frequently used maintenance immunosuppressive drug in ITx including in the *Edmonton protocol*[32].
Mycophenolate mofetil (MMF)	MMF is a prodrug of mycophenolic acid (MPA) which inhibits de novo synthesis of guanosine nucleotides through potent type II inosine monophosphate dehydrogenase (expressed in activated lymphocytes) inhibition[149]. This enzyme is a rate-limiting step of the nucleotide synthesis pathway that T and B cells are more dependent on compared to other cell types. Hence, MPA has potent and selective cytostatic effects on lymphocytes[149]. MPA has been shown to induce apoptosis in activated T cells, and the guanosine nucleotide depleting effects decrease the expression of selective adhesion molecules required for lymphocyte recruitment and infiltration[149].

1.4.3 Toxicities of Immunosuppression

The marked progress made in the field of transplantation is not without the development of more potent and selective immunosuppressives. Despite ITx consisting of a smaller mass transplanted compared to whole organ transplants, these recipients have one of the most rigorous immunosuppressive regimens[150]. As such, multiple toxicities have been associated with the lifelong use of these agents. The chronic immune paralysis in ITx that prevents alloreactivity has been shown to have minor but common risks including mouth ulcers, diarrhea, and acne[151]. More life-threatening risks associated are the development of malignancy and serious infection, although these are rare[151]. Frequently used immunosuppressives (tacrolimus and sirolimus) have also been shown to have direct toxic effects on beta-cell function and survival, thus being inadvertently diabetogenic[152,153]. These multiple toxicities associated with chronic immune suppression remain a major barrier to improving the quality of life of ITx recipients. In fact, the recipients' ability to tolerate these toxicities is a factor in the patient inclusion criteria. The need to reduce or even completely abolish the requirement for chronic immunosuppression is one of many major hurdles (**Figure 1.2)**. Therefore, investigators strive to develop systemic immunosuppressive-free transplant approaches that can be applied to novel and promising extrahepatic transplant sites to effectively subvert the immune response.

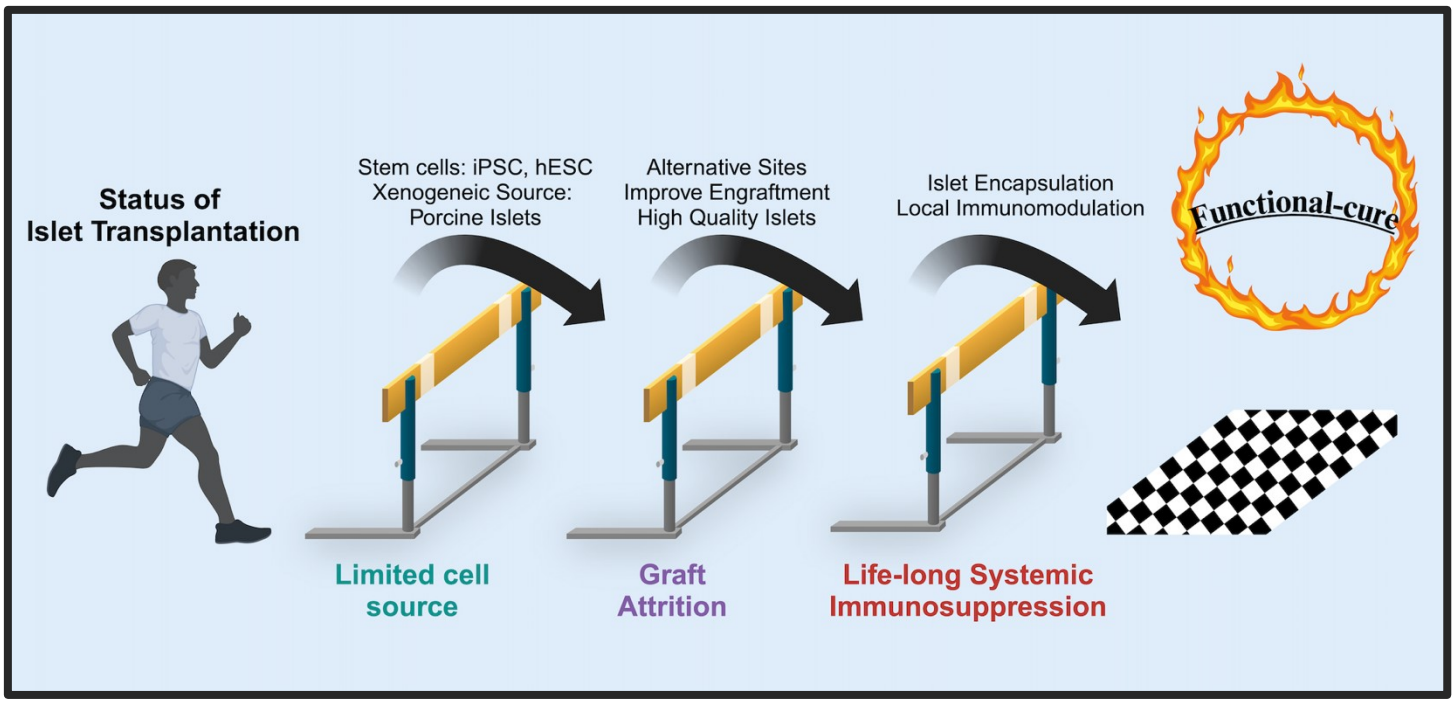


Figure 1.2: Current hurdles in islet transplantation and solutions that overcome these limitations to provide a 'functional-cure' for T1DM. Figure created by Jordan Wong with BioRender and adapted from Desai, 2018[154].

1.5 Future Prospects: Improving Islet Transplantation Outcomes

1.5.1 Devices and Alternative Transplant Sites

While transplanting islets within the liver currently accounts for virtually all clinical ITx and is an effective means that frees recipients from insulin injections, the procedure often results in acute islet cell death and/or gradual graft attrition due to multiple factors in the intraportal hepatic site[155]. Consequently, an estimated ~70% loss in initial islet mass occurs, meaning that recipients routinely require multiple organ donors to achieve and sustain insulin independence[155]. Though effective, ITx into the liver is not the ideal transplant site after considering the hostile nature of the hepatic microenvironment. These considerations previously explored indicate that the liver is not the optimal site for ITx.

To promote engraftment, an ideal ITx site should provide adequate vascularization, substantial space to accommodate for the significant volume of transplanted islets, and sufficient nutrients to aid in islet survival and revascularization. Additionally, avoidance of acute graft loss due to host inflammatory reactions in the peri-transplant period is paramount for decreasing the number of islets required to ameliorate hyperglycemia and can increase the number of T1DM recipients that may be treated. As mentioned, an accessible site would also allow for safety monitoring, non-invasive transplantation, and routine biopsies. Identifying such a site would enable easy retrieval which is ideal for removing abnormal growths associated with alternative cell sources such as insulin-producing stem cells. Considering this all, there are concerted efforts to identify an alternative transplant site.

A multitude of investigators have explored more favourable extrahepatic ITx sites in experimental models and for some in a clinical setting. Experimental sites explored include the liver, spleen, kidney subcapsular space, bone marrow, omentum, peritoneum, intestinal wall,

muscle, subcutaneous space, and immune-privileged sites (anterior chamber of the eye)[156]. While many of these sites effectively cured hyperglycemia in experimental animal models, translating these successes into a clinical setting remains challenging. For instance, islets allografts infused into the bone marrow of non-diabetic rats effectively produced insulin and had limited rejection[157]. Further, ITx in an allogeneic diabetic mouse model demonstrated that bone marrow infusion was superior to the hepatic site in achieving normoglycemia[158]. On the contrary, a pilot randomized controlled clinical trial found that all but 1 patients who received an intra-bone marrow islet infusion (n=7) saw a loss in islet graft function within the first 4 months post-transplantation[159]. These findings are one of many instances where experimental successes fail to translate to clinical outcomes. Coughlan et al. outlines each site for their ability to satisfy the essential characteristics for ITx that were listed above, and the current status of preclinical and clinical findings[156].

The subcutaneous space is a promising extrahepatic site for ITx due to its minimal invasiveness, ability to support a large transplant volume or device, and potential for monitoring transplant function[160-162]. Despite these benefits, the subcutaneous site is a poorly vascularized location, contributing to a hypoxic environment and thus islet cell apoptosis. The majority of vascularized connective and supportive tissue surrounding islets are lost during islet isolation[24]; therefore delivering islets in devices have been explored to recapitulate the endogenous pancreas, promoting engraftment and survival in the subcutaneous space[163]. To achieve such a feat, Barkai's group designed a bioartificial pancreas (*Beta-Air* from *Beta-O2 Technologies Ltd.*) that suspends islets in an alginate hydrogel with sufficient oxygen (via a refillable gas chamber and gas permeable membrane), an external barrier providing immune protection, and a mechanically protective frame[163]. Their subcutaneous implanted device was able to reverse diabetes for up to 6

months in streptozotocin-induced diabetic rats using allogeneic islets[163]. These exciting results led to a clinical trial where 4 diabetic patients underwent subcutaneous implantation with 1-2 of their bioartificial pancreas, however, outcomes were not as triumphant[164]. All four patients saw no changes in metabolic control and low levels of circulating C-peptide, but transplanted islets within the device survived up to 3-6 months post-implantation[164]. However, the recovered bioartificial pancreases demonstrated insufficient *ex vivo* function, as there were low glucose-stimulated insulin responses[164]. Another undesirable outcome seen in these patients was the signs of a substantial foreign-body reaction, indicated by immune cell accumulation in the surrounding areas of implantation[164]. This lifelong complex and dynamic process involves continuous protein adsorption, immune and proinflammatory cell recruitment, and extracellular remodeling which can all contribute to the failures associated with subcutaneously transplanted biomaterials[165]. Hence, alternative strategies that do not utilize permanently implanted devices (a trigger of the foreign-body response) whilst still promoting early vascularization have been heavily explored. This necessary step can involve preconditioning the subcutaneous site with implanting biomaterials such as angiogenic growth factor-loaded polylactide capsules[166], methacrylic acid copolymer coated biomaterial[167], or vascular access catheter[168] that are subsequently removed prior to islet transplantation. Pepper et. al developed a 'device-less' approach that harvests the foreign-body response, demonstrating that pre-implanting and then later removing catheters (at 4-weeks post-implant) sufficiently vascularizes the subcutaneous ITx site[169]. Subsequent syngeneic ITx into this preconditioned site effectively reverses diabetes in mice (>100 days) with a marginal number of islets, while diabetic mice that underwent subcutaneous transplantation without any preconditioning failed to achieve normoglycemia[169]. This promising and novel 'device-less' transplant modality is currently being explored as of 2021, in a phase I clinical trial

consisting of 5 patients with T1DM (ClinicalTrial.gov Identifier NCT05073302). Another method that also promotes early angiogenic growth, but does not require preconditioning the subcutaneous site, was designed by Nalbach et al. where they fused islets to microvascular fragments[170]. This combination was seen to highly enhance in vitro angiogenic activity and effectively restore normoglycemia with a subtherapeutic number of microvascular-fused islets transplanted within the dorsal skin fold of diabetic mice[170]. A similar subcutaneous approach that delivered a bioabsorbable methacrylic acid bounded polymer with islets supported graft revascularization and survival[171]. Though, the application of bioabsorbable materials may also support prevascularization as Kuppan et al. effectively vascularized a subcutaneous site with a nanofibrous polymer scaffold functionalized with angiogenic factors, promoting the survival and function of porcine islets that were later transplanted in mice[172]. Although these strategies show promise, there has yet to be an established alternative islet transplant site used in the clinical setting. Moreover, patients would still be subject to chronic immunosuppressive toxicities regardless of transplant location. Further experimental and clinical progress in novel subcutaneous transplant modalities may one day lead to such an extrahepatic ITx site that can overcome the barriers explored.

1.5.2 Biomaterial Strategies - Localized Immune Modulation

Localized immune modulation is an attractive alternative and a potential replacement for systemic immunosuppression. Targeting immune and inflammatory cells exclusively at the transplant site could help overcome off-target debilitating toxicities associated with chronic systemic immunosuppression. In this framework, researchers utilize two main approaches i) islet encapsulation to prevent contact with immune cells, and ii) localized and sustained release of immunomodulatory agents[173]. If these methods prove effective, there is a high possibility that the

requirement for extensive systemic immunosuppression will be abolished **(Table 1.1 & 1.2)**. Adapting natural and/or synthetic biomaterials has been rigorously investigated.

1.5.2.1 Islet Encapsulation

The current strategies that utilize biomaterial-based islet encapsulation include macroencapsulation, microencapsulation, and nanoencapsulation, which are characterized based on islet-to-host distance[174]. These approaches all work as a physical barrier that protects transplanted islets from immune cell attack, while still enabling them to identify changes in blood glucose levels and subsequently secrete insulin into the circulation. Moreover, these biomaterials must allow for sufficient diffusion of nutrients, oxygen, and metabolic waste, promoting islet survival. The macroencapsulation approach houses the largest number of islets within devices and has the largest islet-to-host distance. Thus, a main disadvantage of this approach is the limited diffusion of oxygen and nutrients which can be detrimental to graft viability and function[175]. To overcome these drawbacks, devices such as the bioartificial pancreas (Beta-Air) employ a gas chamber that provides exogenous oxygen to the islets[163]. Apart from the experimental success that was previously discussed, where diabetic rats achieved long-term normoglycemia with allogeneic islets implanted with Beta-Air devices[163], immunosuppressive-free xenotransplantation in diabetic NHP utilizing this marcoencapsulation technology also demonstrated sustained graft function for up to 6 months[176]. Another macroencapsulation approach that overcomes some challenges with diffusion is intravascular devices. These are hollow semi-permeable fiber devices that house islets within the lumen and directly connects them to host arteries[175]. The semipermeable membrane effectively protects islets from immune-mediated damage, but the blood-device interaction can bring rise to blood coagulation[175]. To avoid excess thrombosis, Song et. al designed an intravascular device using

silicon nanopore membranes and demonstrated superior in vivo hemocompatibility, pore size selectivity, and hydraulic permeability to typical devices that employ polymer membranes[177].

The microencapsulation approach involves encapsulation of a single or small cluster of islets within microcapsules. These strategies minimize the islet-host distance and their often spherical configuration allows for greater diffusion (larger surface area to total volume ratio) relative to macroencapsulation devices[175]. Conformal coating has been an ideal approach, but the risk of incomplete shielding and islet antigens breaching the capsule barrier leaves grafts susceptible to immune attack. The most used microencapsulation materials that are capable of forming spherical capsules around islets are hydrogels, a crosslinked three-dimensional network that can be derived from a wide array of natural and synthetic polymers[174,175]. Extensive polymer modifications promote crosslinking that improves viscoelasticity, hydrophilicity, and shape retention within an aqueous environment of the body, thereby making hydrogels a biocompatible material that can mirror endogenous tissue[178]. Furthermore, their semi-permeable membrane permits oxygen, nutrients, and waste exchange from encapsulated islets, while still protecting against immune infiltration and activation[163]. In terms of islet microencapsulation, researchers have investigated a variety of natural and synthetic derived hydrogels: alginate[179], agarose[180], collagen[181], polyvinyl alcohol (PVA)[182], poly(lactic-co-glycolic acid) (PLGA)[183], etc. These hydrogel varieties show promise in animal models of diabetes, effectively conferring immune protection and prolonging the viability and functionality of transplanted allogeneic islets[179,180,182,183]. The experimental success of microencapsulation-mediated immune cloaking of islets has given rise to clinical trials exploring immunosuppressive-free alternative cell approaches. Although no clinical trials transplanting microencapsulated xenogeneic or allogeneic islets have demonstrated excellent long-term metabolic control[184], a modest reduction in

exogenous insulin usage and hypoglycemic episodes was seen in patients transplanted with microencapsulated porcine islets without immunosuppression[58].

The thinnest barrier, and thus the closest distance between the islets-to-host, is the nanoencapsulation approach. This strategy encapsulates each individual islet with a nano thin coating. By significantly minimizing the coating thickness, diffusional distance also decreases and hence improves islet responsiveness to glucose fluctuations and increases oxygen, nutrients, and insulin diffusion[175]. Furthermore, permeability can be simply modified by controlling coating thickness and composition, in comparison to the other encapsulation approaches that require larger alterations, i.e., altering membrane size, number of islets encapsulated, or encapsulation material[185]. There are two main methods for islet nanoencapsulation: 'PEGylation' and layer-by-layer (LBL) assembly. The former method involves a cell surface modification with poly(ethylene glycol) (PEG), a synthetic polymer that can be modified with chemical groups (acrylates), enabling the formation of crosslinked bioinert networks around islets[186]. This technique, termed 'PEGylation', covalently attaches PEG to islet surfaces with aims to improve biocompatibility via enhancing hydrophilicity, decreasing direct protein adhesions (preventing complement and coagulation cascade), and cloaking islets from immune attack. However, complete immune-blocking effects were not seen in the diabetic NHP model as transplantation of PEGylated allogeneic islets failed to restore euglycemia, even in conjunction with immunosuppressives[187]. The alternative nanoencapsulation approach, LBL assembly, has shown more promise. As the name implies, LBL assembly involves the deposition of alternating nano thin films on an islet surface. The ease of altering the biomaterials and the number of deposited layers used provides researchers the ability to tune and optimize the nanoencapsulated structures. For example, Zhi et. al demonstrated that 8 chitosan/alginate bilayers provided

superior in vivo immune protection of allogeneic islets compared to 4 bilayers in mice, revealing a relationship between structure thickness and immune isolation abilities[188]. In terms of biomaterials used, researchers have explored the incorporation of immunomodulatory materials in these bilayers to locally subvert the immune response. Dr. Hubbert M. Tse's group in Alabama generated an LBL assembly multilayer coating with tannic acid (TA), a natural immunomodulatory polyphenol, and poly(N-vinylpyrrolidone) (PVPON), a biocompatible non-toxic polymer[189]. The formation of hydrogen bonds between these two distinct layers increases structural stability and coating retention around islets. Further, in NOD mice, the TA/PVPON nanoencapsulation approach exhibited reduced in vivo immune cell infiltration, pro-inflammatory chemokine synthesis, and significantly delayed allo- and auto-immune rejection compared to nonencapsulated islets[189]. These thrilling findings may spearhead further developments, possibly inspiring the use of more potent and specific immunomodulatory agents that target negative regulators pathways of T cell immune function (etc., cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4), programmed death 1 (PD-1)). Further, the adaptability of the LDL assembly approach can underpin a multilayer nanocoating with more than 2 distinct layers, further enhancing localized immunosuppression.

1.5.2.2 Alternative Methods for Localized Immune Modulation

These strategies enable clinicians to fine-tune local drug release, which they can adapt based on the period of engraftment. ITx recipients typically require a larger dose of anti-inflammatory or immunosuppressives in the peri-transplant period, later tapering off to lower doses for long-term usage. To accommodate these fluctuating dose requirements, exploring the use of a temperature-dependent elastin-like peptide (ELP), developed by Kojima and Irie, to locally deliver drugs at the site of ITx may serve as a promising solution[188]. This technology

could allow for greater control in local drug administration through locally changing temperatures at the transplant site (given its feasibility) which would trigger drug release. Devices that house islets and contain a refillable drug reservoir can also provide the opportunity for a controlled localized drug release. The Neovascular Implantable Cell Homing and Encapsulation (NICHE) device, developed by Paez-Mayorga et al., precisely fulfills such a role and effectively restored euglycemia in a T1DM rat model[190]. Another alternative method that can support the transplant site microenvironment is co-transplanting islets with cells that secrete immunomodulatory cytokines[191]. Cells explored for this approach include mesenchymal stem cells[192], Tregs[193], Sertoli cells[194] and dendritic cells[192]. However, there are limiting factors to this approach including the source of these immunomodulatory cells and their requirement for long-term survival and function.

1.5.2.3 Drug-eluting Biomaterials

The local and sustained release of immunosuppressives and anti-inflammatories is a strategy that can enhance engraftment, promote tolerance, and improve ITx success by creating less hostile microenvironments. Rather than functionalizing a barrier that confers protection from immune cell contact, this approach typically permits cell infiltration which aids engraftment and islet vascularization. Thus, locally targeting immune infiltrating cells is desirable and can be achieved with synthetic scaffolds, nanoparticles, and microparticles along with cell-based strategies[195,196]. Furthermore, in comparison to systemic immunosuppression, this approach that confines drug release at the site of transplant most likely can achieve a higher local concentration with less off-target toxicities. If this notion holds true, local drug-releasing technology may have a larger therapeutic window that can enable clinicians to amplify desirable immunosuppressive and anti-inflammatory effects.

To achieve localized immunomodulation, synthetic materials have been heavily explored due to material homogeneity and ease of structure fabrication and modification, contributing to a reliable and controlled system[197]. The following are commonly used FDA-approved synthetic materials utilized experimentally for drug delivery in ITx: poly(lactic-co-glycolic acid) (PLGA), polylactide co-glycolide (PLG), poly(ethylene glycol) (PEG), and poly(vinyl alcohol) (PVA)[195,196,198]. These biodegradable materials gradually deteriorate within the host system, releasing a sustained mass of agents that were incorporated within these structures. The most explored forms of this emerging ITx approach include layered scaffolds that entrap drugs, or spherical micro- and nan-particles that encapsulate chemical agents within. Liu and colleagues explored the scaffold delivery approach via implanting streptozotocin-induced diabetic mice with an allogeneic islet-containing multilayered microporous PLG scaffold impregnated with transforming growth factor-beta 1 (TGF-ß1), an immunomodulatory cytokine[199]. In vitro drug kinetics of TGF-ß1, found within layers of the scaffold, demonstrated a burst initial release in the first 3 days (>90% total mass)[199]. Implantation of this scaffold within the epididymal fat pad decreased inflammatory cytokine (TNF-α, IL-2, monocyte chemotactic protein-1) production by at least 40%, corresponding to a 60% drop in leukocyte infiltration and significant delay in allograft rejection compared to empty scaffolds[199]. Another group, utilizing a polydimethylsiloxane scaffold to deliver fingolimod (FTY720, Gilenya), an immunosuppressive that inhibits effector T cell recruitment and migration[200], demonstrated a similar in vitro burst release with the bulk of the drug being released in the first 7 days[201]. However, no significant improvements were seen when this scaffold was implanted in the epididymal fat pad of diabetic mice[201]. The length of drug release could be an explanation for varying levels of experimental success with scaffold drug delivery. As discussed, in clinical ITx, recipients are required to

endure lifelong immune suppression to prevent allo- and auto-immune graft rejection. Within this framework, a burst initial drug release may not be effective at subverting the immune response long-term, thereby failing to achieve prolonged graft function. Hence, researchers have explored alternative methods for achieving a longer sustained local drug release.

Synthetic drug-eluting particles are a promising technology to achieve sustained local immunomodulation. As mentioned, PLGA is a biodegradable and FDA-approved synthetic material that has typically been employed for controlled drug delivery[198]. Drug-eluting micelles fabricated from PLGA or other polymers have also been utilized in other therapeutic applications including chemotherapy, ocular and neurological drug delivery, and vaccines[202]. As such, the popularity of applying this promising technology to ITx has increased over the years. In terms of maintenance immunosuppression in ITx, a longer sustained release is ideal; researchers have explored polymer-based microparticles (MP) as they typically elute drugs slower and for a longer duration than their smaller counterpart, nanoparticles[203]. Recently, Kuppan et al. developed such a system where they encapsulated dexamethasone (dex), a systemically diabetogenic and anti-inflammatory glucocorticoid, in PLGA MP[204]. Their formulation eluted dex in vitro for at least 30 days and co-localizing these MPs with allogeneic islets transplanted under the kidney capsule of diabetic mice resulted in a two-fold increase of recipients that achieved euglycemia for 60 days post-transplantation compared to empty MP controls (both groups received a short course of CTLA-4-Ig injections to block of T-cell costimulation)[204]. Furthermore, they also saw significantly reduced proinflammatory cytokine expression and increased Treg localization within the grafts of the dex-MP treated recipients[204]. Other groups have explored the use of sustained immunosuppressive drug-eluting MPs in the context of experimental ITx. Pathak's group fabricated PLGA encapsulated tacrolimus (FK506), a

commonly used maintenance immunosuppressive in clinical ITx (**Table 1.2)**, and co-delivered this biotechnology with xenogeneic islets within the subcutaneous space of streptozotocin-induced diabetic mice[205]. By 30 days post-transplant, the mice that received these FK506-eluting MP were euglycemic (60% survival), while mice transplanted with islets alone all became hyperglycemic by day 15[205]. An alternative MP system, formulated by Fan et. al in Singapore, draws on polycaprolactone (PCL) and PLGA polymers to fabricate two distinct types of rapa-eluting MPs that release drugs at different rates[206]. Combining these two particles allowed them to have an initial burst release, via porous PCL MPs, followed by a sustained PLGA MP-mediated rapa release for up to 30 days in vitro[206]. Rapa is another maintenance immunosuppressive that is widely used in clinical ITx (**Table 1.2)**, and co-transplanting this drug-eluting MPs system with allogeneic islets within the anterior chamber of the eye demonstrated a modest (10 day) delay in graft rejection compared to MP containing no drugs[206]. Conversely, nanoparticles' tendency to burst release may be ideal for certain tolerance induction strategies[203]. Bryant and colleagues demonstrate this clinically attractive approach by utilizing PLG nanoparticles to deliver donor antigens intravenously, inducing long-term donor-specific tolerance for allogeneic islets transplanted in diabetic mice[207]. Despite these limited results highlighting the potential of nano- and micro-particles in subverting the immune response, further investigation into these approaches in the context of ITx is necessary. Optimizing this technology may one day lead to a replacement of chronic systemic immunosuppression where ITx recipients would only require occasional MP administrations.

1.6 Objective, Outline, and Hypothesis

The many hurdles still present in the field of clinical ITx are apparent, though there are thrilling novel solutions to address these limitations to bring the procedure one step closer to becoming a mainstay treatment option for T1DM (**Figure 1.2)**. Along with these efforts described previously, the thesis aims to add to this body with a primary focus on localized immunomodulation. Overall, the objective of the thesis was to develop a localized drug-eluting system that subverts the immune response at the islet allograft site, effectively reducing or abolishing the need for chronic systemic immunosuppression. Our lab employed a biomaterial approach in an attempt to achieve such a feat. The research presented in Chapter 2 highlights our work developing and characterizing rapa-eluting microparticles (MP) fabricated from PLGA. Next, we examined the application of rapa-MP to preserve islet allograft function with distinct preclinical transplant models.

In Chapter 2, the objectives were to achieve by the following: i) Rapa-MP fabricated with a modified single-emulsification technique and characterized in vitro and in vivo, ii) Calculated mass of rapa within MP to determine a therapeutic dose of rapa-MP for transplantation, iii) Examine the cellular function of islets in vitro exposed to the therapeutic rapa-MP dose, thus providing insight on islet toxicity, iv) Syngeneic ITx model to examine in vivo islet toxicity of rapa-MP therapy, v) A multitude of allogeneic ITx models (human and murine islets) used to characterize the type of tolerance generated by rapa-MP while examining it as a mono- or combination therapy, and vi) Gene expression and cytokine analysis studies to postulate cellular mechanisms involved with islet allograft tolerance. Through these sets of experiments, hypotheses can be examined with precision and findings may provide insights on clinical translatability.

Broad application of beta-cell transplantation for T1DM is significantly hindered by the rigorous immunosuppression required, and the ability to tolerate chronic use dictates recipient selection. To address this major hurdle in ITx, the project herein examined the *central hypothesis that localized delivery of rapa via novel FDA-approved PLGA MP will subvert the immune responses and prolong islet allograft function in mice*. Overall, we demonstrate that the tolerance generated by the rapa-MP is suspected to be islet-graft-specific leading to failure of donor-matched skin grafts. Furthermore, we expected the murine islet allografts treated with rapa-MP to have a downregulation of immunogenic gene expression at the transplant site compared to control grafts. Finally, in our humanized mouse model proof-of-concept, in which recipients were co-transplanted with human islets + rapa-MP and engrafted with human peripheral blood mononuclear cells, we anticipated a lower islet graft infiltration with leukocytes to control grafts indicating the translational potential of our localized immunomodulatory approach.

1.7 Summary

To date, T1DM still afflicts many individuals worldwide and has been established as an autoimmune-driven disease. Although the mainstay treatment of exogenous insulin injections can help T1DM patients achieve normoglycemia, the daunting risk of life-threatening hypoglycemia unawareness remains interconnected. Islet transplantation has been established as an effective means of reducing these events and granting recipients a period freed from insulin injections; limitations in the field include poor cell survival in the hepatic site, limited donor supply, toxicities of chronic systemic immunosuppression, and long-term deterioration of graft function. Thus, the procedure is typically restricted to those who suffer from brittle diabetes. Nevertheless, the status of clinical ITx today is not without the ground-breaking progress made

in the field ever since the first islet tissue transplant in 1893. Further advancements in validating more favourable extrahepatic transplant sites, alternative cell sources, and biomaterial-based localized immunomodulation can help surmount the barriers explored. Herein we examined the ability of rapa-eluting MP to preserve islet graft function, effectively reducing the requirement for chronic systemic therapy.

Chapter 2: Exploring Local Immune Modulation with Rapamycin-Eluting Microparticles to Preserve Islet Graft Function in Mice

2.1 Introduction

Islet transplantation (ITx) is an attractive strategy to restore glycemic control in those living with T1DM. Despite providing a 'functional-cure' through recapitulating physiological insulin delivery, ITx has not become a first-line treatment option due to procedural and functional limitations. Parallel to other organ transplants, the shortage in islet supply acts as the initial limiting factor to the procedure. Immediate and gradual graft attrition proves another barrier that typically requires patients to receive a multi-donor islet infusion. While ITx may grant recipients a period freed from insulin injections, insulin independence is not sustained long-term: 61% of patients were insulin independent at 1-year post-transplant, dropping to 20% at 10-years in 255 transplants at a single centre[35]. Up to 70% of islets fail in the first 24 hours after transplantation, majorly driven by the instant blood-mediated inflammatory reaction (IBMIR), a portal vein site-specific complication[80-82]. In addition, the gradual graft failure can be attributed to the allo- and auto-immune response in combination with off-target toxicities of drug therapies. Lastly, the requirement for toxic and lifelong systemic immunosuppressive therapy adds a significant barrier, and in fact, the ability to sustain treatment dictates patient inclusion. As such, ITx is reserved for those with brittle T1DM. Herein, we explore localized drug delivery of rapamycin (rapa, sirolimus) to abate the toxicity associated with systemic therapy.

Rapa is a potent maintenance immunosuppressive frequently used in islet and solid organ transplantation. Through selective inhibition of the mammalian target of rapamycin complex 1 (mTORC1), rapa effectively prevents allograft rejection by impairing dendritic cell maturation and function, and inhibits T cell and B-cell proliferation[146]. Moreover, rapa has been seen to

promote the expansion of functional regulatory T cells (Tregs) in T1DM patients[148], reducing the occurrence of autoimmunity. Certainly, systemically delivered rapa can facilitate overimmunosuppression, predisposing patients to infection and neoplastic growths beyond other off-target toxicities. Phase III clinical trials examining rapa safety in renal transplantation found hyperlipidemia and thrombocytopenia as the most common adverse outcomes[208,209]. Importantly, especially in the context of ITx, several studies have reported an increased occurrence of new-onset diabetes in renal[210-212] and cardiac[213] transplant patients on rapa. While the diabetogenic mechanism is still unknown, insulin resistance and islet toxicity are thought to play a role. Numerous studies show the direct toxicity of rapa on beta-cell function[152]. Hence, local rapa delivery must employ a system that is controlled to enable sustained drug release within a therapeutic and non-toxic range.

The application of biomaterials in the clinical setting can provide favourable controlled outcomes, notably with localized immunomodulation. Poly(lactic-co-glycolic acid) (PLGA) is a Food and Drug Administration-authorized biocompatible polymer that provides the ability for sustained drug release due to its biodegradable properties[214]. Broad utilization of PLGA has been explored as the capacity to tune chemical and physical properties can alter biodegradability, and thus drug delivery[198]. Strategies that encapsulate agents into polymer-derived particles achieve sustained delivery in areas including vaccines, hormone therapy, and chemotherapy[202]. Our previous work encapsulating dexamethasone and cyclosporine A into PLGA microparticles (MP) demonstrated the feasible and effective application of this technology in ITx[215,216]. However, long-term function and tolerance were not achieved in these studies. Hence our efforts in refining our approach with rapa, a more potent immunosuppression.

This project aimed to develop a localized rapa-eluting system to support islet allograft survival, engraftment, and function while reducing or abolishing the need for systemic immunotherapy. With the use of PLGA, rapa was encapsulated into MP and the efficacy in generating islet graft tolerance was examined in a fully major histocompatibility complex-mismatch murine ITx model. The additive or synergistic effects of our rapa-MP were tested in combination with acute and low-dose CTLA-4-Ig systemic therapy. Promise in either domain, as a mono- or combination therapy, underpins the notion of a long-term systemic 'immunosuppressive-free' ITx approach.

2.2 Materials and Methods

2.2.1 Formulation of Rapa-Eluting and Empty PLGA Microparticles

A modified single oil-in-water emulsion technique was used to evaporate solvent as previously described [204,215,217]. In brief, 20 mg of rapamycin (rapa, L C Laboratories, MA, USA) and 200 mg of poly(lactic-co-glycolic acid) (PLGA, Sigma-Aldrich, ON, Canada) were dissolved in dichloromethane (DCM, Sigma-Aldrich, ON, Canada). The solution was then added dropwise to 10 ml of cold 4% polyvinyl alcohol (PVA, 80% hydrolyzed, Mw 9000-10000, Sigma-Aldrich, ON, Canada) with a magnetic stirrer (VWR International, ON, Canada) set at maximum speed for 5 min. Next, the solution was added dropwise to 200 ml of a 2% PVA solution at room temperature while being mechanically stirred (Caframo, BDC6015, ON, Canada) at 1000 rpm for one hour. Upon completion, rapa-MP were left to gravity-settle at 4 °C overnight and then collected via centrifugation (Allegra® X-15R centrifuge, Beckman Coulter, NH, USA) at 3000 rpm for 5 min at 4 °C. Lastly, the MP were lyophilized (Dura-Dry™ MP,

FTS Systems, NY, USA) and stored at 4 °C for later use (**Figure 2.1A**). Empty (drug-free) PLGA MP were formulated in the same steps described, apart from adding rapa in the mixture. These empty MP were used as the negative controls.

2.2.2 In Vivo and In Vitro Characterization of Rapa-Microparticles

Visualization and characterization of rapa-MP were completed via scanning electron microscopy (ZEISS EVO 10 Scanning Electron Microscope, Zeiss, NY, USA). Imaging was completed on lyophilized rapa-MP coated with gold sputtering (Hummer 6.2 sputter coater, LADD Research Industries, VT, USA) and mounted on carbon tape. To quantify encapsulation efficiency, 10 mg of lyophilized rapa-MP (n=6) was dissolved in acetonitrile (Sigma-Aldrich, ON, Canada): methanol (Fisher Scientific, ON, Canada) mixture (8:2) and rapa concentration was analyzed with high-performance liquid chromatography (HPLC) (Agilent Technologies, 1200 series, CA, USA). The column concentration was set to 35 °C, the rate of elution was 0.2 mL/min, and the rapa was detected at 278 nm. The unknown concentration of rapa (dissolved from the MP) was extrapolated from a curve generated by a series of known rapa concentrations (10 – 1000 ug/mL) ran in parallel.

The in vitro release kinetics of rapa-MP were collected over a 35-day period. Briefly, 30 mg of rapa-MP (n=3) were mixed into 1 mL of phosphate-buffer saline (PBS, Thermo Fisher Scientific, ON, Canada) and left in a water bath set to 37 °C. At each collection point, 200 µl of the supernatant was collected for analyses and replaced with a fresh 200 µl PBS. Concentration was determined with a Multiskan SkyHigh microplate spectrophotometer (Thermo Fisher Scientific, ON, Canada) and analyzed at 278 nm**.** To determine the unknown sample

concentrations, samples were extrapolated off the curve generated by a series of rapa standards (10 – 100 ug/ml) ran in parallel.

The in vivo rapa release kinetics were examined in naïve C57BL/6 mice implanted with a 4.0 mg rapa-MP and collagen type I (Corning, NY, USA) mixture under the kidney capsule. The kidney containing the rapa-MP was removed at t=0, 1, 3-, 7-, 14-, and 21-days following implantation, homogenized, and analyzed with HPLC with a similar method performed for rapa-MP encapsulation efficiency.

2.2.3 Assessing the Bioenergetics of Islets Co-Cultured with Rapa-Microparticles In Vitro

The Extracellular Flux Analyzer XF24 (Seahorse Bioscience, Agilent, North Billerica, MA, USA) was used to examine the bioenergetics, mitochondrial potency and oxygen consumption rates of human islets treated with rapa-MP or rapa. Human islets were obtained from HumanIslet Core (Alberta Diabetes Institute, Edmonton, AB, CA) and treated for 24 h with either 1 mg rapa-MP (n=3), 2 mg rapa-MP (n=3), or 25 nM rapa (n=3), and a control group (n=3). A mesh screen was used in all groups to separate islets from the MP in Connaught Medical Research Laboratories (CMRL-1066, Mediatech, Manassas, VA) supplemented with fetal bovine serum (10%), L- glutamine (100 mg/L), penicillin (112 kU/L), streptomycin (112 mg/L), and HEPES (25 mmol/L) at pH 7.4. Following treatment, 70 islets were handpicked per well and plated onto the XF24 analyzer as per the manufacturer's direction. Modified Agilent Seahorse XF Assay Media (Dulbecco′s Modified Eagle′s Medium/Ham′s Nutrient Mixture F12 (DMEM), Seahorse Bioscience, Agilent, North Billerica, MA, USA) supplemented with 1% FBS, sodium pyruvate, L-glutamine, and 2.8 mM glucose was used to in to plate the islets and make the following solutions. Oxygen consumption rate was monitored for a total of 265 min

and sequentially exposed to the following: 1) baseline 2.8 mM glucose, 2) 16.8 mM glucose, 3) 5 μM oligomycin, 4) 3 μM FCCP (Carbonyl cyanide-p-trifluoromethoxyphenylhydrazone), and 5) 5 μM antimycin A/rotenone. Each agent modulates distinct components of the mitochondrial electron transport chain, and by examining differences in respiration at each phase, we determined the efficiency of energy metabolism. The oxygen consumption rate was normalized to baseline respiration. Human islet studies were performed with the approval of the Human Research Ethics Board at the University of Alberta (Pro00092479). Donor information can be found in **Supplementary Table 1.**

2.2.4 Animal Care, Transplant Studies, and Graft Monitoring

2.2.4.1 Animal Care and Islets Isolation

All animal handling and studies were performed following the Canadian Council of Animal Care and guidelines provided by the Institutional Ethical Committee at the University of Alberta (AUP00002977). Mice were housed in a pathogen-free and sterile environment with access to *ad libitum* of water and pelleted food. Syngeneic and allogeneic studies had donors of 8–12 weeks old male and female BALB/c mice (The Jackson Laboratory, Bar Harbor, ME, USA) that weighed 22-27 g. Islets were prepared through isolation and purification, following a methodology that was previously described[168]. In brief, a solution of ice-cooled collagenase (5 mg/mL) and thermolysin (0.2 mg/mL) (LiberaseTM TL Research Grade, Roche Diagnostics, Mannheim, Germany) in Hank's Balanced Salt Solution (HBSS, Sigma-Aldrich, ON, Canada) was injected through the common bile duct to distend the pancreas *in situ* with simultaneous blockage of the duct entering at the duodenum. Next, the mice pancreata were digested at 37 °C for 14 min in a shaking water bath (Thermo Fisher Scientific, ON, Canada) at 50 rpm. With

histopaque density gradient (1.108, 1.083, and 1.069 g/mL, Sigma-Aldrich, ON, Canada) centrifugation at 3000 rpm for 12 min, islets were purified from the solution. Islets were collected and washed with HBSS, cultured in CMRL supplemented with fetal bovine serum (10%), L- glutamine (100 mg/L), penicillin (112 kU/L), streptomycin (112 mg/L), and HEPES (25 mmol/L) at pH 7.4 for one hour, then used in transplantation.

2.2.4.2 Diabetes Induction

Induction of diabetes in recipient mice was facilitated through intraperitoneal (i.p.) injections with 180 mg/kg streptozotocin (STZ, Sigma-Aldrich, ON, Canada) reconstituted in acetate buffer (pH 4.5). Male and female BALB/c and C57BL/6 mice (The Jackson Laboratory, Bar Harbor, ME, USA), between age 8-12 weeks, served as recipients. Following at least two consecutive days of non-fasted blood glucose (BG) readings > 18 mM on a OneTouch UltraMini glucose meter (LifeScan, Burnaby, BC, Canada), recipients were considered diabetic and utilized in ITx studies within one week.

2.2.4.3 Islet Transplantation and Graft Monitoring

Methodology of ITx under the kidney capsule followed work that was previously described, utilizing polyethylene tubing and centrifugation to deliver pelleted islets with MP[168]. Syngeneic ITx studies were conducted by transplanting a low dose (350-400 islets) of BALB/c islets into STZ-induced diabetic BALB/c recipients. With islets, recipients were either co-transplanted with 1 mg (0.1 mg/kg, n=3) or 2 mg (0.2 mg/kg, n=6) of rapa-MP. The allogeneic ITx studies followed a similar methodology, with a larger dose of islets (500-550 islets) and diabetic C57BL/6 serving as recipients. In the allogeneic study, two groups received a short course of 10 mg/kg cytotoxic T-lymphocyte associated protein 4 immunoglobulin (CTLA-4-Ig,

Biocell, West Lebanon, NH) administered i.p. at 1 hour before transplant, and 2, 4, and 6 days following. Groups transplanted are as follows: 1) 1 mg of empty MP + islets (n=4), 2) 0.1 mg/kg rapa-MP + islets (n=6), 3) Empty MP + islets + CTLA-4-Ig injections (n=8), 4) 0.1 mg/kg rapa-MP + islets + CTLA-4-Ig injections (n=6). In both ITx studies, the recipient's non-fasted BG levels were monitored three times weekly with a OneTouch UltraMini Glucose meter. Graft failure or allograft rejection was indicated by two consecutive readings > 18 mM, and the graft was collected with the recipient being euthanized thereafter. An intraperitoneal glucose tolerance test (IPGTT) was conducted at 35- or 100-days post-transplant in euglycemic syngeneic and allogeneic ITx recipients, respectively. Mice were fasted overnight and injected with a 3 g/kg bolus of glucose (DMVet, Coaticook, QC, Canada) i.p. with BG measured at 0, 15, 30, 60, 90 and 120 minutes. Glucose clearance was compared through an area under the curve (AUC) analysis of the BG readings. Finally, graft-bearing nephrectomies were completed to confirm graft function.

2.2.4.4 Skin Transplantation

Skin transplant studies were completed on euglycemic ITx allograft recipients at 75 d post-transplant to characterize the tolerance generated by the treatment conditions[218,219]. Two groups of mice were transplanted with skin grafts: 1) Diabetic C57BL/6 + 500 BALB/c islets + 0.1 mg/kg rapa-MP + CTLA-4-Ig (n=9), and 2) Naïve C57BL/6 (n=10). Mice were either transplanted with a donor-matched BALB/c skin graft (Group 1: n=5, Group 2: n=5) or a third-party C3H skin graft (Group 1: n=4, Group 2: n=5) (**Figure 2.7)**. All mice were given a C57BL/6 skin graft to confirm procedural success. Briefly, ~1 cm^2 of the donor-matched (BALB/c) or third-party (C3H) flank skin was transplanted at the left flank, and C57BL/6 skin to the right flank (**Figure 2.9)**. Next the skin grafts were secured with pressure bandages for 8 days.

Monitoring of skin grafts occurred at 8-15 days via digital photography with a ruler and examined for rejection. Measurements with the ruler helped assess the proportion of skin remaining and the area was calculated from the digital photographs. Skin grafts were considered rejected when less than 10% of viable tissue remained (< 0.1 cm^2 tissue). BG monitoring of ITx recipients was ongoing through the skin transplant study.

2.2.4.5 Humanized Mouse Model

Humanized mouse model experiments were conducted on male and female non-diabetic *NOD* scid *gamma(NSG)*-MHC I/II double knockout (DKO) mice transplanted with human islets (IsletCore, Alberta Diabetes Institute, Edmonton, AB, CA) under the kidney capsule following the same methodology described above. Since these mice lack MHC Class I and II they are less susceptible to graft versus host disease, facilitating us to examine the ability of rapa-MP to subvert the human immune system, once reconstituted. Mice were either co-transplanted with 1 mg of empty MP (n=8) or 0.1 mg/kg rapa-MP (n=9), and 1500 IEq of human islets. One hour following transplantation, mice were injected i.p. with 40×10^6 human peripheral blood mononuclear cells (PBMC, STEMCELL Technologies, Vancouver, BC, CA). Grafts were collected at 3- and 6-w (3w: empty MP, n=3; rapa-MP, n=3) (6w: empty MP, n=5; rapa-MP, n=6) post-transplant for immunohistochemistry analysis, and tail vein blood was also collected at these timepoints and t=0 to confirm reconstitution with human immune cells was successful (analyzed via flow cytometry). Spleens were also collected at the time of graft retrieval as an additional confirmation of successful reconstitution and analysis was completed with flow cytometry and immunohistochemistry. Human islet studies were performed with the approval of the Human Research Ethics Board at the University of Alberta (Pro00092479). Islet*s* and PBMC donor information can be found in **Supplementary Table 1.**

2.2.5 Islet Graft Gene Expression and Immune Cell Analysis

2.2.5.1 Intragraft Gene Expression

Extraction of RNA was performed using the RNeasy Micro Kit (Qiagen, Hilden, Germany) as per the manufacturer's directions. Grafts collected from diabetic C57BL/6 recipients co-transplanted with BALB/c islets and the four groups tested in the allogeneic transplant study were collected 7 d post-transplant for analysis. NanoString nCounter Mouse Immunology panel (NanoString Technologies, WA, USA) was used to assess the expression of 547 genes annotated across 32 signalling pathways ranging from immune and inflammatory cell activation, cytokine signaling, and host-pathogen interaction. Panel probes (capture and report) and 200 ng of RNA were hybridized overnight at 65 °C for 16 h. Samples were scanned at maximum scan resolution capabilities (555 FOV) using the nCounter Digital Analyzer. Quality control of samples, data normalization, and data analysis were performed using nSolver software 4.0 (NanoString Technologies, WA, USA).

2.2.5.2 Flowcytometric Analysis of Immune Cells

Flowcytometric analysis was performed on peripheral blood and single-cell splenic suspension in the humanized mouse model study with non-diabetic NSG-MHC I/II DKO mice transplanted with human islets and reconstituted with human PBMCs. Briefly, using a sterile scalpel blade, 1 mm of tail tissue was clipped, and 100 µl of blood was collected into 5 mL of red blood cell (RBC) lysis (155 mM NH_4Cl, 10 mM $KHCO_3$ and 2 mM EDTA in DPBS) buffer by gently milking the tail. RBC lysis was performed by incubating the peripheral blood in RBC lysis buffer for 30 minutes at room temperature with constant shaking. Dissected spleens were filtered through 40 µm nylon mesh membranes, washed with PBS, and red blood cells lysed was performed

as above. Single-cell suspensions were washed with PBS and fixed with 4% paraformaldehyde on ice for 30 minutes. For long-term storage, PFA was removed, and single cells were stored in PBS at 4 °C. Samples were permeabilized and stained using the BD Cytofix/Cytoperm Fixation/Permeabilization Solution Kit (BD Biosciences cat. 554714) as per manufacturer instructions. Primary antibodies were incubated for 1 h on ice with an antibody cocktail containing subset-specific antibodies in **Supplementary Table 2**. Murine cells were identified and excluded from analysis by staining with a monoclonal antibody specific to murine CD45. Nuclear permeabilization was performed using the theTrue-Nuclear™ Transcription Factor Buffer Set (BD Biosciences cat. 424401) as per manufacturer instructions. Nuclear staining was performed for 1 h on ice. Cells were resuspended in fluorescence-activated cell sorting buffer (2% (v/ v) FBS, 2 mM EDTA in DPBS) and kept on ice until flow cytometry acquisition and analysis. At least 50,000 events were acquired using the CytoFLEX S flow cytometer. Isotype controls and fluorescence minus one control were used to accurately gate positive staining and data were and analyzed using the CytExpert software (Beckman Coulter).

2.2.5.3 Immunohistochemistry

Formalin-fixed human islets grafts collected at 3 and 6 w post-transplant from NSG-MHC I/II DKO mice reconstituted with human PBMCs were processed and sectioned (5 μm thickness) from paraffin blocks. Tissue slides were rehydrated, and antigen was retrieved, prior to being blocked with 20% normal goat serum (Jackson ImmunoResearch, Pa, USA) for 1 h. Next, slides were incubated for 1 h with primary antibodies of anti-guinea pig α-insulin (1:5, Agilent, CA, USA) and washed 3 times with 1x PBS. Following the wash, tissue slides were incubated for 1 h with the following secondary antibody and subsequently washed as described above: anti-guinea pig Alexa fluor 488 (1:200, Invitrogen, MA, USA). Lastly, tissue samples

were coverslipped with 100 µL of DAPI anti-fade reagent (Thermo Fisher Scientific, ON, Canada) and left to dry in the dark at room temperature. To examine CD45 cell infiltration, tissue slides were similarly rehydrated, antigen retrieved and blocked as described above following incubation with rabbit monoclonal anti-CD45 (1:350, Abcam, CAM, UK) overnight at 4 °C. Following incubation, slides were washed 3 times with 1x PBS then incubated with biotinylated goat anti-rabbit (Thermo Fisher Scientific, ON, Can- ada) for 1 h. Slides were visualized with Widefield Fluorescence Microscope Colibri (Zeiss, NY, USA) and Whole Slide Scanner Axioscan Z1 (Zeiss, NY, USA). CD45+and insulin cells were automatically quantified with QuPath.

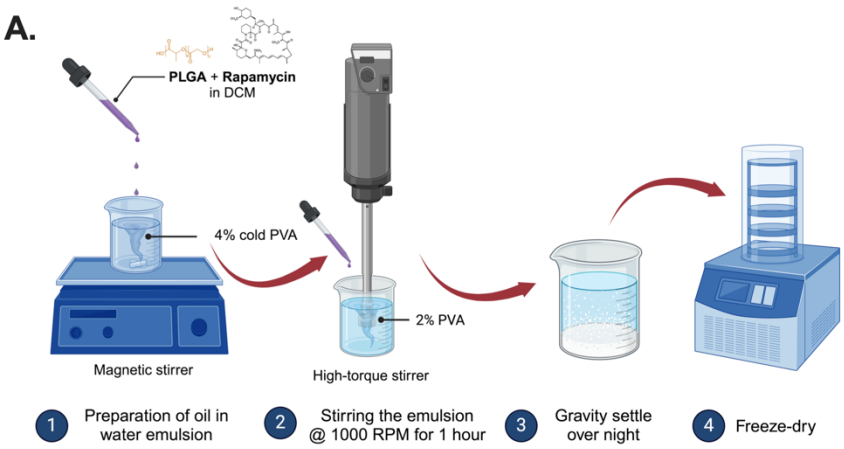
2.2.6 Statistical Analysis

Data are represented as mean ± standard error of the mean (SEM) and analyzed with GraphPad Prism 9 (GraphPad Software, La Jolla, Ca, USA). In vivo and in vitro experiments comparing multiple conditions were executed via a one-way ANOVA with Tukey's post-hoc test for multiple comparisons. Examining cohort differences in IPGTT experiments was conducted with an unpaired t-test. Kaplan-Meier survival and euglycemia curves were compared with Mantel-Cox (log-rank) testing. NanoString nCounter gene expression data was analyzed and represented as a heatmap, gene set analysis scores or 2 (-ΔΔCT) using GraphBio and GraphPad Prism version 9.3.1.

2.3 Results

2.3.1 In Vitro and In Vivo Characterization of Rapa-Microparticles

Rapa-MP were formulated by a single emulsification technique with mechanical stirring **(Figure 2.1A)**. These lyophilized microparticles were spherical, uniform, and smooth when examined under SEM (**Figure 2.1C).** Encapsulation efficiency of 87.3 ± 0.84 % was determined from analysis of 10 mg lyophilized rapa-MP **(Figure 2.1B).** Gradual and sustained rapa release was observed in vitro over 35 days, with 39.4 ± 0.35 % total drug eluted at this timepoint **(Figure 2.1D)**. In comparison, an immediate rapid release of rapa was seen in the in vivo release studies. One day following delivery, 30.7 ± 4.6% of rapa was released. The following days showed a more sustained release pattern as a cumulative 79 ± 4.7% of rapa was released at 21 days. The initial burst seen may be attributed to rapa incorporated on the surface of the microparticles dissolving, while the remaining drug was loaded internally and gradually hydrolyzed. To determine the weight of rapa-MP to transplant, we calculated the mass of rapa-MP required to deliver 0.1-0.2 mg/kg rapa per day for the span of 30 days, assuming all drug is released at that period. Using data generated from HPLC analysis, we determined 0.1 mg/kg of rapa per day dosing in a 30 g mouse as 1 mg of MP (0.1 mg/kg rapa-MP) and 0.2 mg/kg as 2 mg of MP (0.2 mg/kg rapa-MP) (**Figure 2.1E)**. The target of 0.1 mg/kg rapa was targeted as it was determined as subtherapeutic in ITx with previous work using systemic administration[220]. The subsequent experiments examine these doses of MP.



B.

Sample ID	Rapamycin Concentration in 10 mg rapa-MP (µg/mL)	Encapsulation efficiency (%)
RAPA-MP_S1	904.78	90.47
RAPA-MP_S2	874.26	87.42
RAPA-MP_S3	877.60	87.76
RAPA-MP_S4	881.57	88.15
RAPA-MP_S5	856.88	85.68
RAPA-MP_S6	844.78	84.47
Average	873.31	87.33
SEM	8.48	0.84

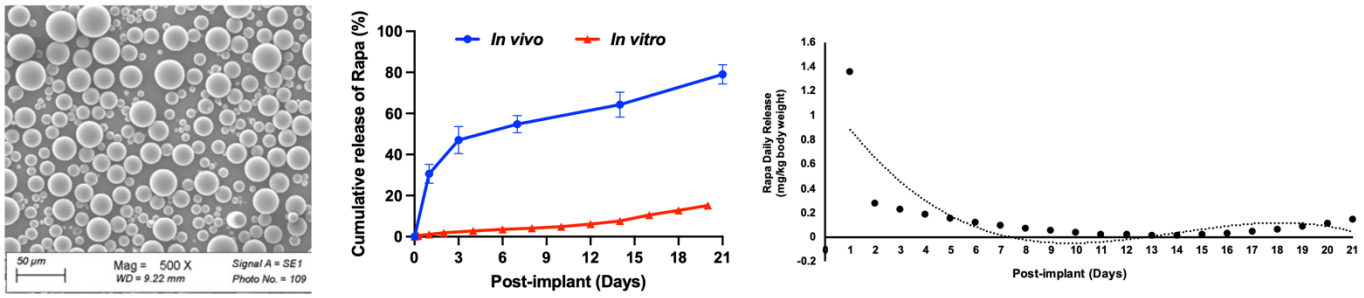


Figure 2.1: Fabrication and characterization of rapa-eluting MPs. **A)** Methodology of the single-emulsion technique adapted to make rapa-MP. **B)** Encapsulation efficiency of rapa examined from 10 mg of rapa-MP. **C)** Scanning electron microscopy (magnification = 500X) of lyophilized rapa-MP coated with gold sputtering. **D)** Cumulative in vitro (red, 30 mg rapa-MP, n=4) and in vivo (blue, 4 mg rapa-MP, n=4 per time-point) release kinetics of rapa from rapa-MP over 21 d with data expressed as mean +/- SEM. **E)** Daily dose of rapa release in a 30 g recipient with the black curve representing the normal distribution.

2.3.2 Examining the Toxicity of Rapa-Microparticles to Islets

The bioenergetics of human islets from one donor were treated for 24 h with rapa and rapa-MP was assessed with a Seahorse XFe24 extracellular flux analyzer **(Figure 2.2A)**. Treatment conditions were islets co-cultured with 1 mg and 2 mg of rapa-MP. The 25 nM of rapa served as a positive drug control and is a concentration titrated in clinical ITx patients. Quantifying the changes in oxygen consumption rate throughout the subsequent injection of electron transport chain modulators provided insight into mitochondrial function and ATP generation **(Figure 2.2B)**. Eight parameters were generated and of note, basal respiration-normalized ATP-linked respiration of islets treated in 25 nM rapa (n=3) was lower than the control (n=3), 1 mg (n=3), and 2 mg (n=3) rapa-MP islets with mean differences of 0.19 ± 0.03 ($p<0.001$), 0.14 ± 0.03 ($p<0.01$), and 0.13 ± 0.03 ($p<0.05$), respectively (**Figure 2.2C**). Moreover, islets demonstrated a higher proton leak in the 25 nM rapa compared to all conditions (control: 0.12 ± 0.02 ($p<0.01$), 1 mg rapa-MP: 0.14 ± 0.02 ($p<0.01$), 2 mg rapa-MP: 0.11 ± 0.02 ($p<0.01$) (**Figure 2.2D)**. Most importantly, there were no significant differences comparing islets either co-cultured with 1 or 2 mg rapa-MP compared to control islets in all eight parameters (**Supplementary Figure 1)**. These experiments were repeated on two additional donors, though were excluded as the positive control failed to generate significant change (**Supplementary Figure 2,3).** These data indicated no apparent toxicity of these doses of rapa-MP in vitro, in which we proceeded to transplant in our in vivo models.

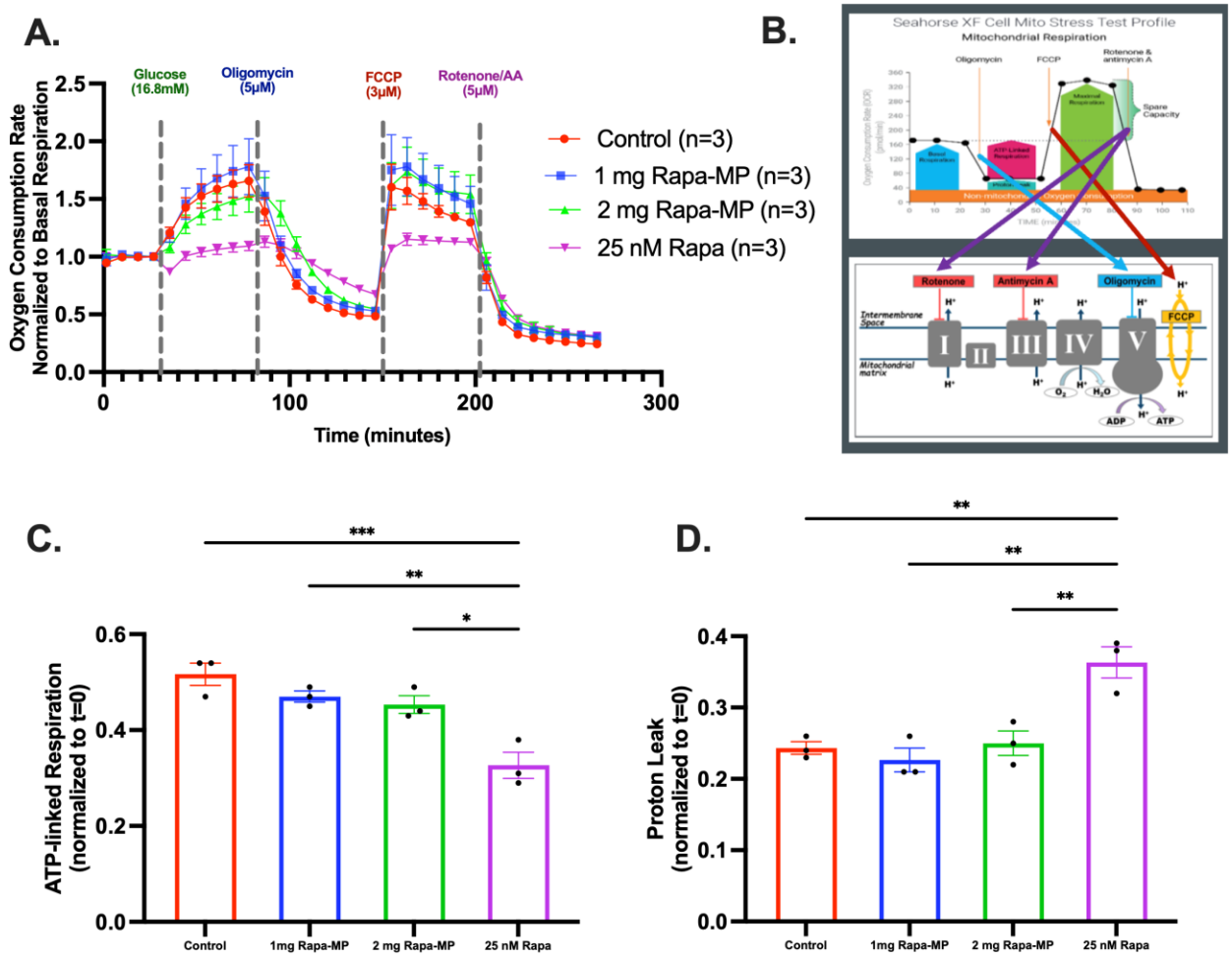


Figure 2.2: Respiration and mitochondrial function of human islets co-cultured for 24 hours with rapa-MP or rapa. All data is represented as mean +/- SEM and normalized to basal respiration. **A)** Oxygen consumption rate of islets following sequential stimulation with glucose and electron transport chain modulators. Dotted grey lines indicate instances of agent injected into the port. **B)** Mechanism of injected agents on the mitochondrial electron transport chain. Images were obtained from the manufacturer (Agilent) and adapted. **C)** ATP-linked respiration extrapolated from the oxygen consumption curve. **D)** Proton leak with higher values indicating inefficient mitochondrial function. *p<0.05, **p<0.01, ***p<0.001

A syngeneic mouse model, where BALB/c islets were transplanted into STZ-induced diabetic BALB/c recipients, examined the direct adverse effects of rapa-MP on islet graft function. The two tolerable doses identified in vitro, 1 mg (n=3, 0.1 mg/kg rapa-MP) or 2 mg (n=6, 0.2 mg/kg) of rapa-MP, were co-transplanted with a marginal dose of islets under the kidney capsule of recipients. All mice transplanted with the lower dose (3/3) achieved and maintained euglycemia to the 42 d endpoint, whereas half (3/6) achieved this in the 2 mg rapa-MP cohort (**Figure 2.3A**). AUC analysis of an IPGTT performed on the euglycemic recipients at 35 d post-transplant was comparable between groups **(Figure 2.4A,B)**. A graft-bearing nephrectomy was performed to confirm graft-dependent euglycemia as all recipients became hyperglycemic thereafter (**Figure 2.3A,B)**. The outcomes of this study demonstrate that 0.2 mg/kg rapa-MP may compromise islet function and the 0.1 mg/kg rapa-MP was tolerated. Subsequent transplant studies employed the lower dose.

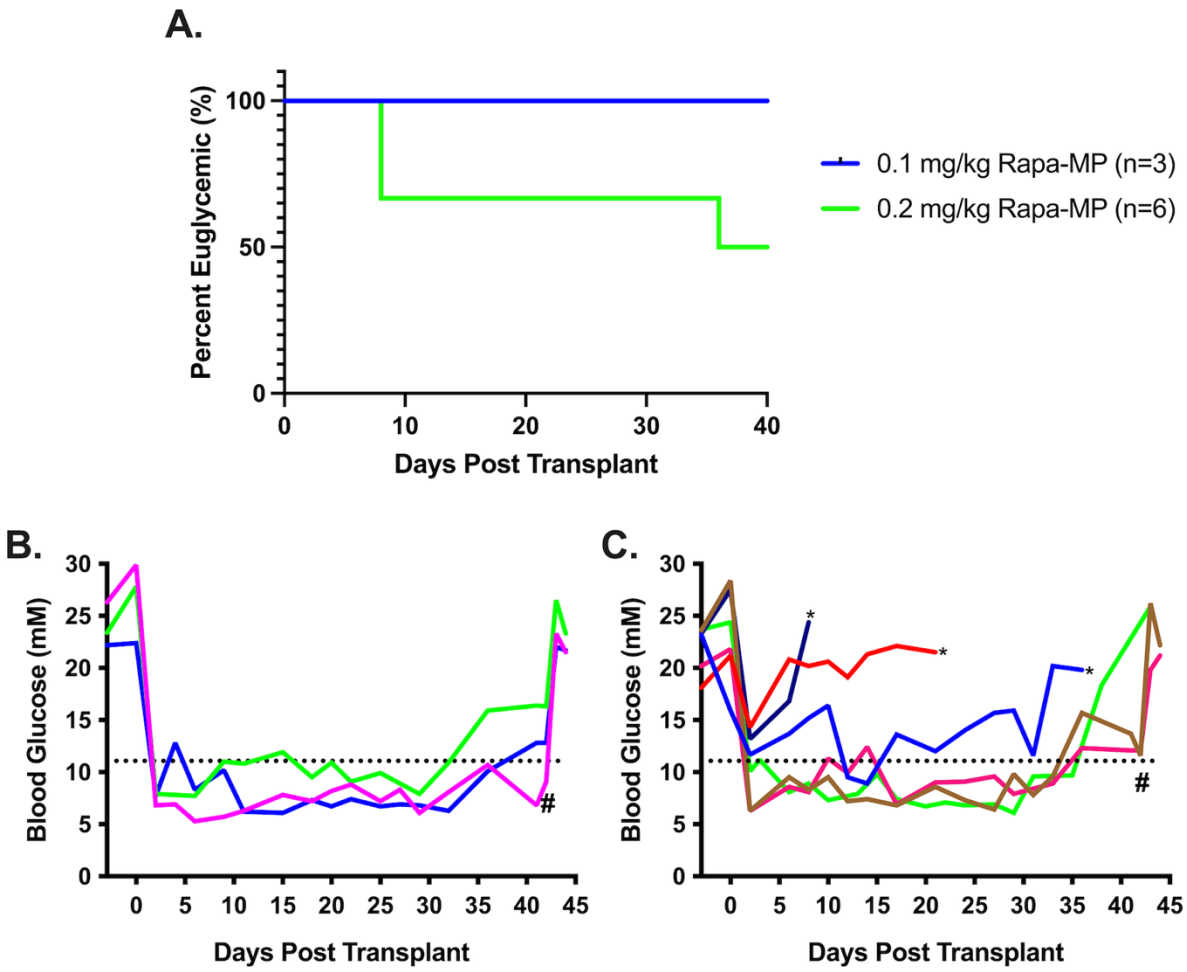


Figure 2.3: Non-fasted blood glucose readings of STZ-induced diabetic BALB/C mice that underwent syngeneic ITx. **A)** Percentage of syngeneic ITx recipients euglycemic **B)** Non-fasted BG of diabetic BALB/c mice co-transplanted with 0.1 mg/kg rapa-MP + 350 BALB/C islets. **C)** Non-fasted BG of diabetic BALB/c mice co-transplanted with 0.2 mg/kg rapa-MP + 350 BALB/C islets. Graft-bearing nephrectomy completed at # to confirm graft-dependent euglycemia. * indicates the early endpoint of recipients, confirmed with two subsequent glucose readings > 18 mM.

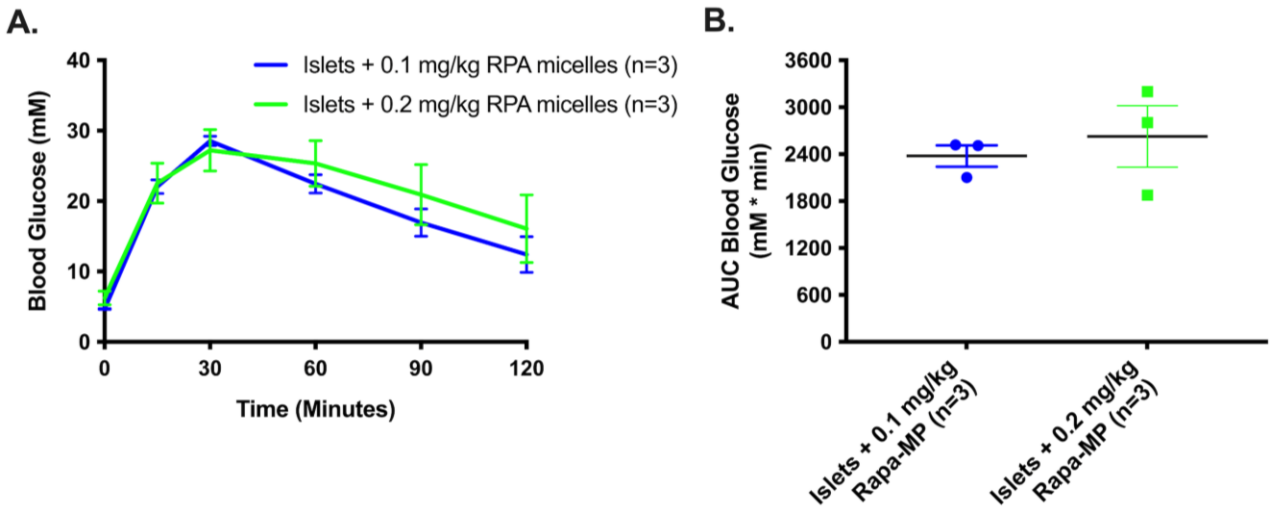


Figure 2.4: 35 d post-transplant intraperitoneal glucose tolerance test (IPGTT) of BALB/c mice co-transplanted with a marginal mass of syngeneic islets and either 0.1 or 0.2 mg/kg rapa-MP. **A)** BG collected over the duration of the IPGTT. **B)** AUC analysis of the BG curve.

2.3.3 Co-transplantation of Rapa-Microparticles Prolong Murine Islet Allograft Function

To assess the immunomodulatory potential of the rapa-MP in islet allografts, a fully mismatched ITx model of minor/major histocompatibility complex (MHC) was utilized. Diabetic C57BL/6 recipients ($H2^b$) co-transplanted with 0.1 mg/kg rapa-MP (n=6) and BALB/c islets ($H2^d$) displayed a significantly longer function than control mice that were co-transplanted with empty MP (n=4) **(**$p<0.01$, **Figure 2.5A**). All the control mice rejected the islet allografts by 19 d post-transplant, while the recipients co-transplanted with 0.1 mg/kg of rapa-MP began rejection at 27 d with 2/6 sustaining euglycemia for greater than 100 d (**Figure 2.5B)**. A dual therapy approach with low-dose and acute CTLA-4-Ig injections + co-transplantation with 0.1 mg/kg of rapa-MP (n=6) led to all recipients sustaining euglycemia beyond 100 d post-transplant (**Figure 2.5A)**. Survival in the dual therapy group was also significantly longer than in mice receiving CTLA-4-Ig + empty MP (n=8), as only 3/8 recipients survived long-term in the latter group ($p<0.05$). Comparison between the dual therapy to the rapa-MP or empty MP groups also showed a significantly longer survival ($p<0.05$ and $p<0.01$, respectively). Furthermore, this dual therapy group displayed a more robust glucose tolerance compared to naïve non-diabetic C57BL/6 mice when faced with a 100 d post-transplant IPGTT (**Figure 2.6A,B)**. This was determined by comparing the AUC analysis of rapa-MP + CTLA-4-Ig treated (2160 ± 67.5, n=6) and naïve (2697 ± 218.8, n=5) mice ($p<0.05$, **Figure 2.6B).** Respectively, the divergence of BG readings were 8.4 ± 2.9 mM and 8.9 ± 2.0 mM higher in naïve mice at 60- and 90-min following glucose injection ($p<0.05$ and $p<0.01$, **Figure 2.6A).** These glycemic outcomes suggested a synergistic effect of the dual therapy approach, as immunomodulation was potentiated when therapies were combined.

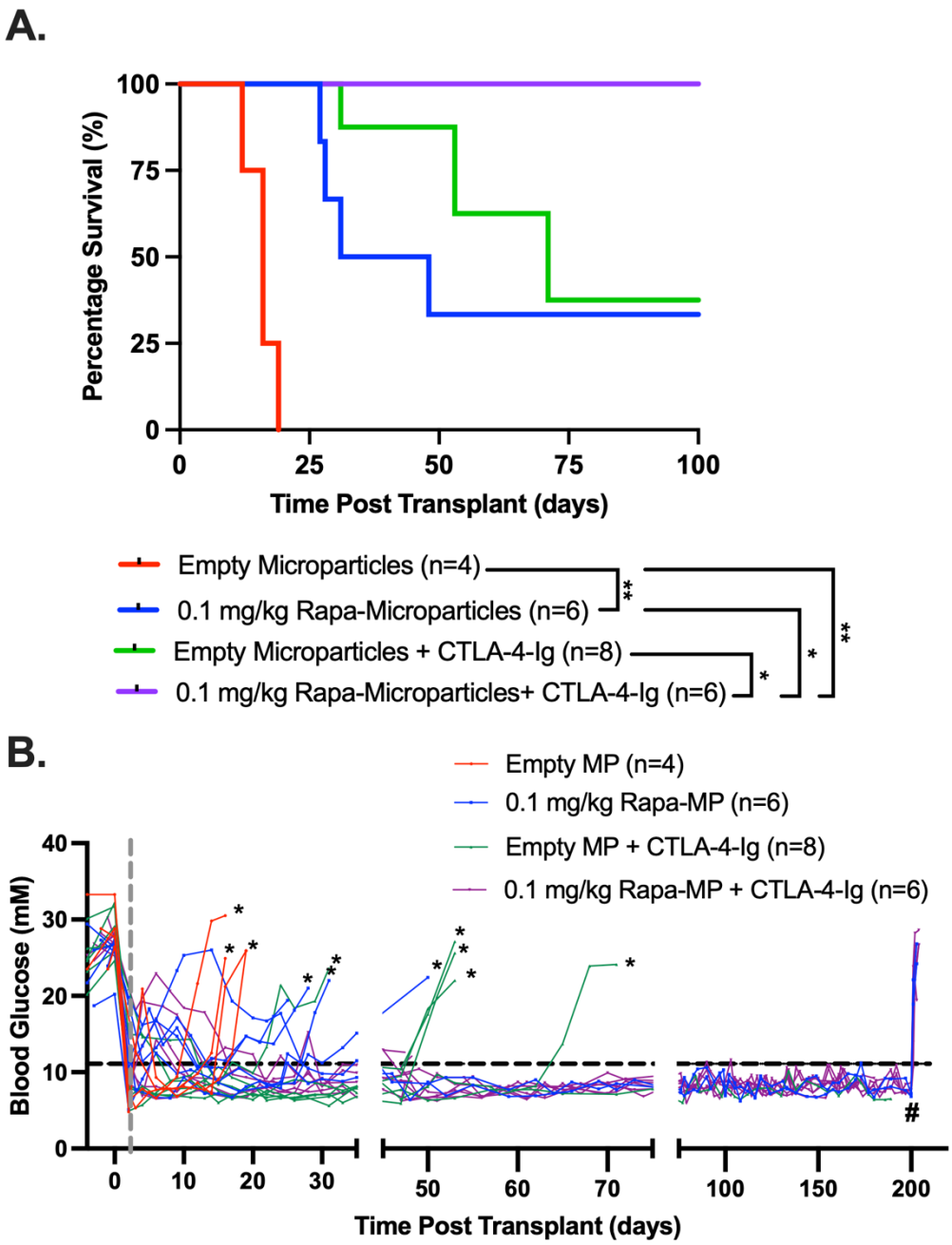


Figure 2.5: Survival and graft function of a murine allogeneic islet transplant model (diabetic C57BL/6 recipients, BALB/c islets donors). Along with islets, mice either received empty-MP (red), 0.1mg/kg rapa-MP (blue), empty-MP + CTLA-4-Ig injections (green), or 0.1 mg/kg rapa-MP + CTLA-4-Ig injections (purple). **A)** Recipient survival to 100 d post-transplant. **B)** Non-fasted BG curve of recipient mice represented in (**A).** * Indicate euthanasia due to persistent hyperglycemia and # represents islet graft removal to confirm graft-dependent euglycemia. *p<0.05, **p<0.01

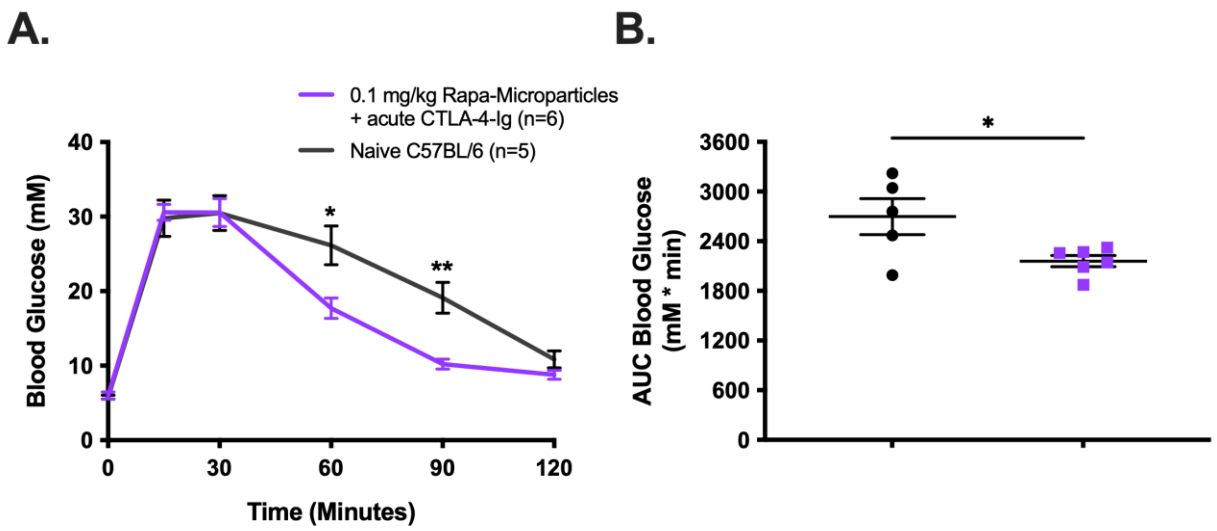


Figure 2.6: 100 d post-transplant IPGTT of ITx recipients that received dual therapy of 0.1 mg/kg rapa-MP and CTLA-4-Ig injections (purple), ran in parallel with naïve C57BL/6 mice (black). **A)** BG readings of mice challenged with an IPGTT. **B)** AUC analysis of the IPGTT curve. AUC and BG timepoint comparison was completed with an unpaired t-test. *p<0.05, **p<0.01

2.3.4 Rapa-MP + CTLA-4-Ig Injection Dual Therapy Induced Operational Tolerance

Skin transplantation studies characterized the type of tolerance generated by diabetic ITx allograft recipients who received the dual therapy and BALB/c islets. Naïve controls (n=10) and the cohort of euglycemic ITx recipients that previously received 0.1 mg/kg rapa-MP + CTLA-4-Ig injections (n=9) underwent skin transplantation with donor match (BALB/c), third-party (C3H) and syngeneic (C57BL/6) full-thickness skin grafts (**Figure 2.7)**. Rejection of the donor-matched skin graft occurred significantly later in ITx recipients at 13.0 ± 0.63d in reference to naïve mice which were completely rejected at 9.4 ± 0.24 d ($p<0.05$, **Figure 2.8A)**. Notably, no difference in third-party skin graft rejection was seen between the two groups (**Figure 2.8B)**. Moreover, third-party skin graft rejection was more rapid, being fully rejected at 9.0 and 10 ± 0.58d for the naïve and ITx recipients, compared to the donor-matched skin graft rejection (**Figure 2.8A,B)**. As suspected, islet allograft failure was generated thereafter in recipients of the donor-matched skin grafts, but not the third-party skin group (**Figure 2.8C,D).** A graft-bearing nephrectomy of the remaining euglycemic mice–the third-party skin graft recipients–confirmed islet graft function as mice returned to hyperglycemia (**Figure 2.8D)**. Simultaneous syngeneic skin transplants confirmed procedural success in all skin transplanted mice (**Figure 2.9)**. Hence, the combination therapy of rapa-MP + CTLA-4-Ig injections was concluded to generate an islet-graft-specific and operational tolerance, as we observed ongoing islet graft function in the absence of immunosuppression throughout the third-party skin graft failure.

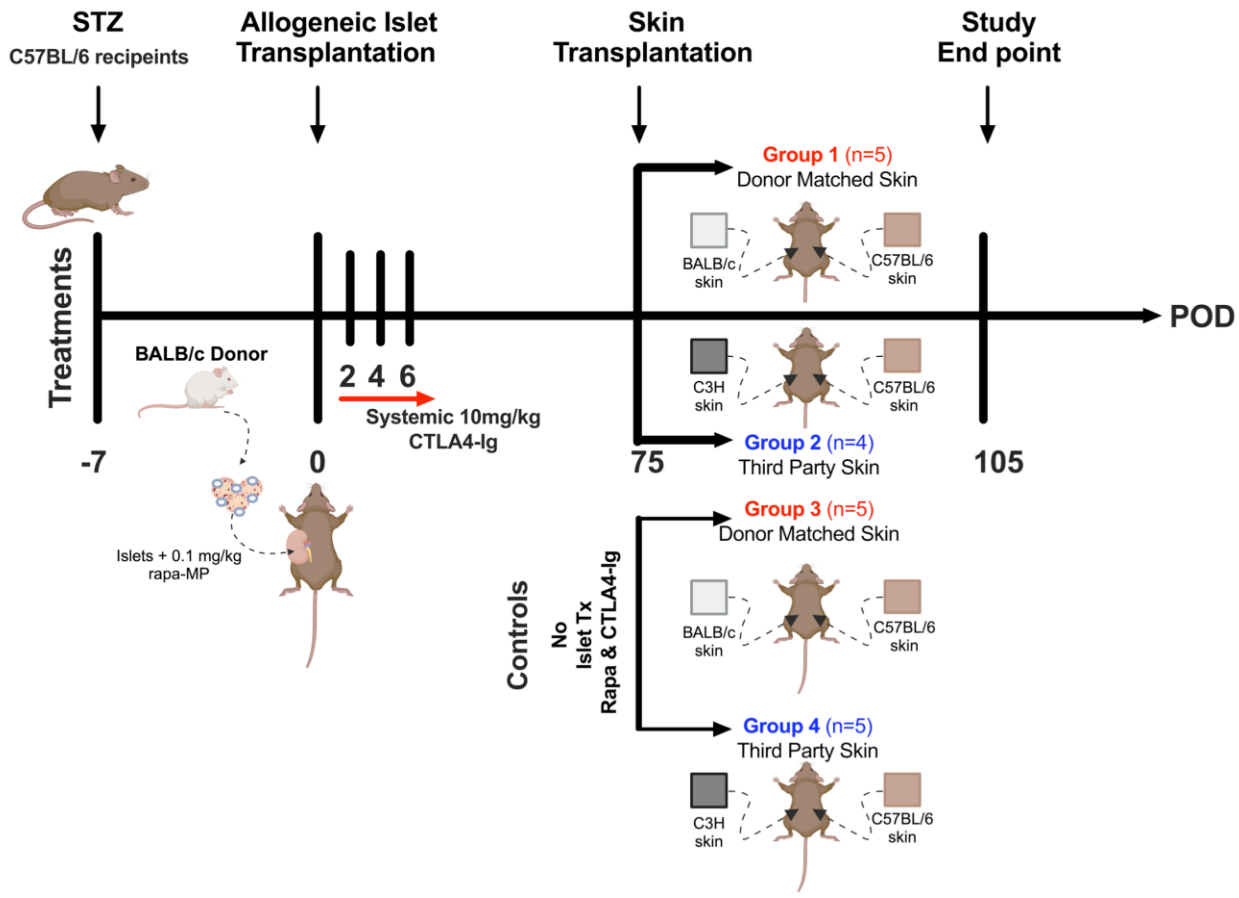


Figure 2.7: Skin transplantation schematic of diabetic C57BL/6 recipients receiving BALB/c islets + 0.1 mg/kg rapa-MP + CTLA-4-Ig therapy. Red text represents donor-matched (to islets grafts) skin transplants and blue represents third-party (C3H mice) skin grafting. Figure made in BioRender by Jordan Wong.

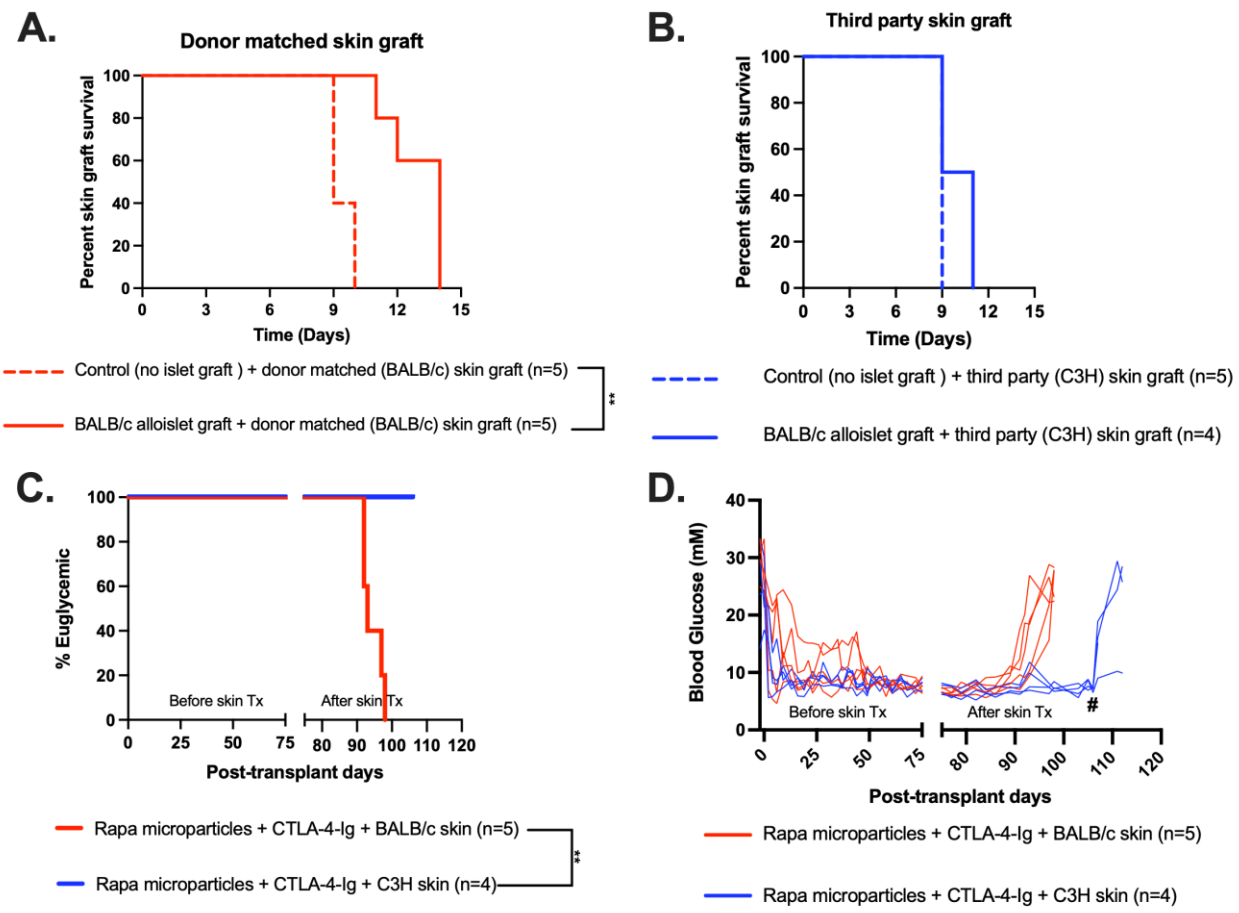


Figure 2.8: Skin graft and islet graft survival of diabetic C57BL/6 recipients receiving BALB/c islets + 0.1 mg/kg rapa-MP + CTLA-4-Ig therapy. Red data represent donor-matched and blue represents third-party skin graft recipients. **A,B)** Rejection of skin grafts with dotted lines representing control naïve mice that underwent skin transplantation. **C)** Percentage of euglycemic ITx recipients through the skin transplant study. **D)** Representation of BG and islet allograft rejection in relation to the timeline of skin transplants (indicated by the break in the x-axis) with # representing graft-bearing nephrectomy. **p<0.01

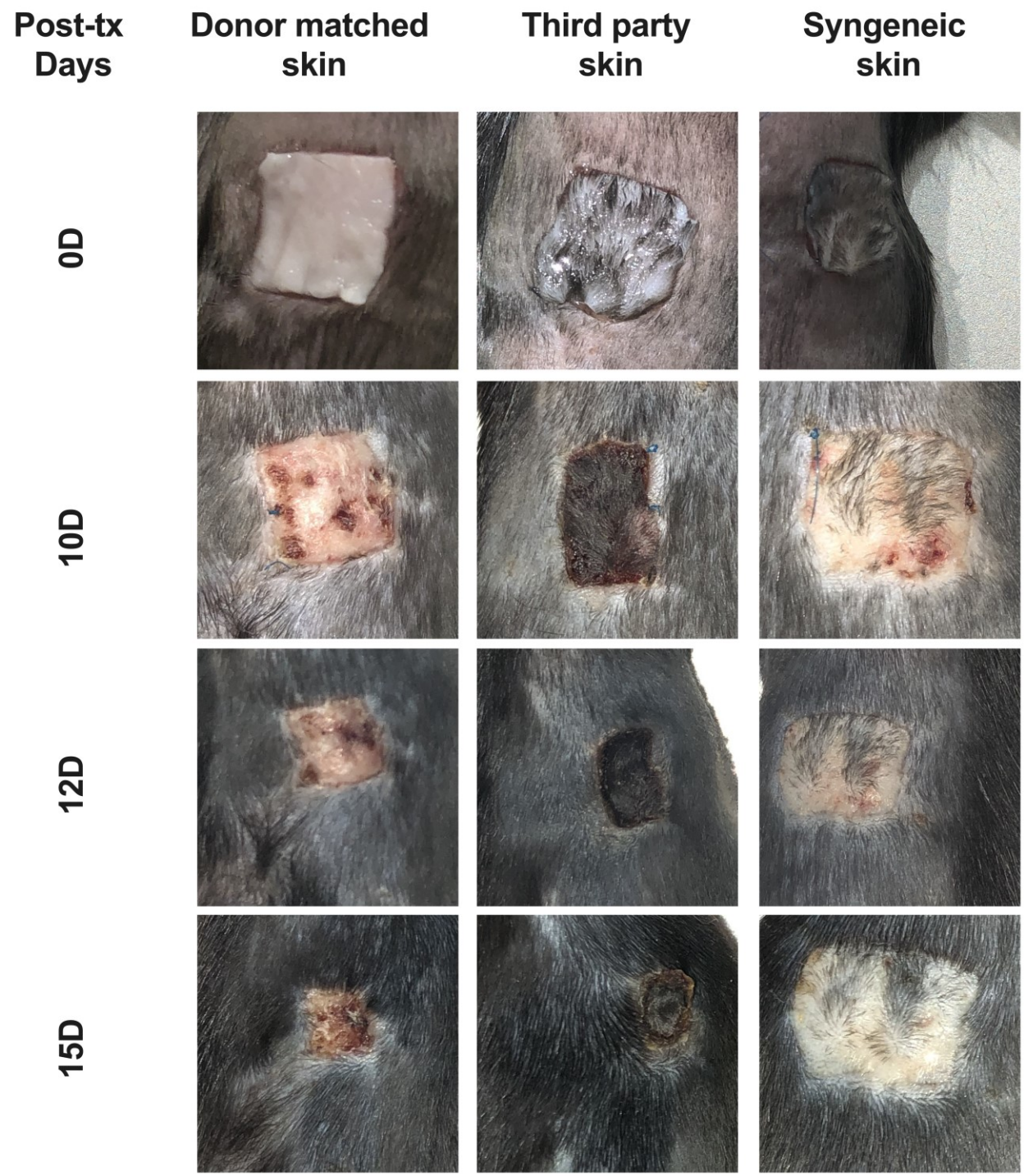


Figure 2.9: Digital photography of different periods of the skin transplantation study. Recipients were C57BL/6 mice and all received syngeneic skin (C57BL/6, right column), and either donor-matched (BALB/c, left column) or third-party (C3H, middle column) skin grafts. Skin grafts were considered rejected when <10% viable tissue remained.

2.3.5 Rapa-MP and CTLA-4-Ig Downregulate Inflammatory, Innate, and Adaptive Immune Pathways in Murine Allogeneic Islets Grafts.

To elucidate the mechanisms involved with the prolongation of murine islet allograft survival and function when treated with rapa-MP and/or CTLA-4-Ig injections, analysis of genes involved with key immune cell, inflammatory, and cell death activation pathways were conducted in the acute period following transplant. NanoString analysis of intragraft extracted RNA collected 7 d post-transplant enabled the comparison of gene expression. Heatmap representation of gene set analysis scores showcased divergences in the transcription of key signaling pathways involved with adaptive and innate immune cell regulation and inflammation of the islet graft microenvironment upon co-transplantation with rapa-MP and/or CTLA-4-Ig injections (**Figure 2.10A)**. These measures represent the average of significance measures across the genes within the respective pathways, calculated from differential expression. Specifically, pathways involved with innate immunity were comparable to empty MP grafts, apart from a lower NLR signaling score in the rapa-MP + CTLA-4-Ig graft (-2.00 ± 0.54) to the empty MP control graft (0.93 ± 0.47) (p=0.0179, **Figure 2.10B-D)**. Comparison of pathways involved in activating adaptive immunity between the empty MP control and rapa-MP + CTLA-4-Ig grafts were more diverse as the latter group had lower adaptive immune system (Control: 2.19 ± 0.79; RAPA + CTLA: -3.56 ± 1.31, p=0.0185, **Figure 2.10E**), lymphocyte activation (Control: 3.25 ± 1.1; RAPA + CTLA: -4.97 ± 1.6, p=0.0056, **Figure 2.10F**), T cell receptor signaling (Control: 1.84 ± 0.45; RAPA + CTLA: -2.64 ± 0.83, p=0.0023, **Figure 2.10G**), toll-like receptor signaling (TLR, Control: 1.32 ± 0.61; RAPA + CTLA: -2.45 ± 0.73, p=0.0260, **Figure 2.10H**), T helper 1 cells differentiation (Th1, Control: 0.88 ± 0.23; RAPA + CTLA: -1.40 ± 0.29, p=0.0005, **Figure 2.10I**), T helper 2 cell differentiation (Th2, Control: 0.70 ± 0.27; RAPA + CTLA: -1.25 ± 0.31, p=0.0064, **Figure 2.10J**), MHC class I (Control: 0.92 ± 0.40; RAPA + CTLA: -1.68 ± 0.51,

p=0.0256, **Figure 2.10K**), MHC class II (Control: 0.74 ± 0.24; RAPA + CTLA: -1.09 ± 0.49, p=0.0270, **Figure 2.10L**), and type I interferon signaling (Control: 1.08 ± 0.30; RAPA + CTLA: -1.94 ± 0.42, p=0.0011, **Figure 2.10M**) scores. These trends were also seen with pro-inflammatory pathways including the nuclear factor kappa-light-chain-enhancer of activated B cells signaling (NFkappaB, Control: 1.25 ± 0.55; RAPA + CTLA: -2.31 ± 0.68, p=0.0171, **Figure 2.10N**), tumour necrosis factor family signaling (TNF, Control: 1.20 ± 0.52; RAPA + CTLA: -2.14 ± 0.59, p=0.0117, **Figure 2.10P**), cytokine signaling (Control: 2.42 ± 1.08; RAPA + CTLA: -4.54 ± 1.4, p=0.0186, **Figure 2.10Q**), chemokine signaling (Control: 1.48 ± 0.52; RAPA + CTLA: -2.62 ± 0.73, p=0.0052, **Figure 2.10R**), and type II IFN signaling (Control: 1.09 ± 0.44; RAPA + CTLA: -2.02 ± 0.61, p=0.0127, **Figure 2.10S**) scores. Grafts that received rapa-MP alone or CTLA-4-Ig + empty MP had comparable significant pathway scores to all other groups for inflammation and innate immunity, though rapa-MP graft had lower pathways scores in adaptive immunity, the T cell receptor signaling (Control: 1.84 ± 0.45; RAPA: -1.65 ± 1.10, p=0.0342, **Figure 2.10G**), and Th1 differentiation (Control: 0.88 ± 0.23; RAPA: -0.70 ± 0.47, p=0.0254, **Figure 2.10I**) scores in comparison to the empty-MP control.

Differential expression of 547 genes and their significance scores were used to compare expression levels in the inflammatory, adaptive, and innate immune pathways. When comparing the three treatment groups with the empty-MP control as a reference, the fold changes in different genes expressed were visually represented in volcano plots (**Figure 2.11**). The grafts that received rapa-MP + CTLA-4-Ig injections had the highest number of significantly downregulated genes (**Figure 2.11C).** Grafts that received rapa-MP followed next, with CTLA-4-Ig + empty MP having the least significant and down-regulated genes to control grafts (**Figure 2.11A,B).** More precisely, compared to controls the rapa-MP + CTLA-4-Ig saw a

downregulation of several genes involved in NFkappaB signaling (*CCL4, LTA,* and *TNF8F11*, **Figure 2.12A**), NLR signaling (*CCL12, CCL5,* and *TNF*, **Figure 2.12B**), and T cell receptor signaling (*CD247, CD3D, CTLA4,* and *CD8a*, **Figure 2.12C**) which represent activating pathways in inflammation, innate, and adaptive immunity, respectively. Some of these genes were also downregulated in the rapa-MP and CTLA-4-Ig grafts compared to empty MP controls. Overall, grafts treated with rapa-MP + CTLA-4-Ig had the strongest and largest impact on the transcriptional regulation of genes involved with inflammation, innate immunity, and adaptive immunity signaling pathways, with rapa-MP grafts following and CTLA-4-Ig + empty MP grafts with the smallest change. As expected, all three groups saw a downregulation of these genes relative to empty MP control grafts, and not an upregulation.

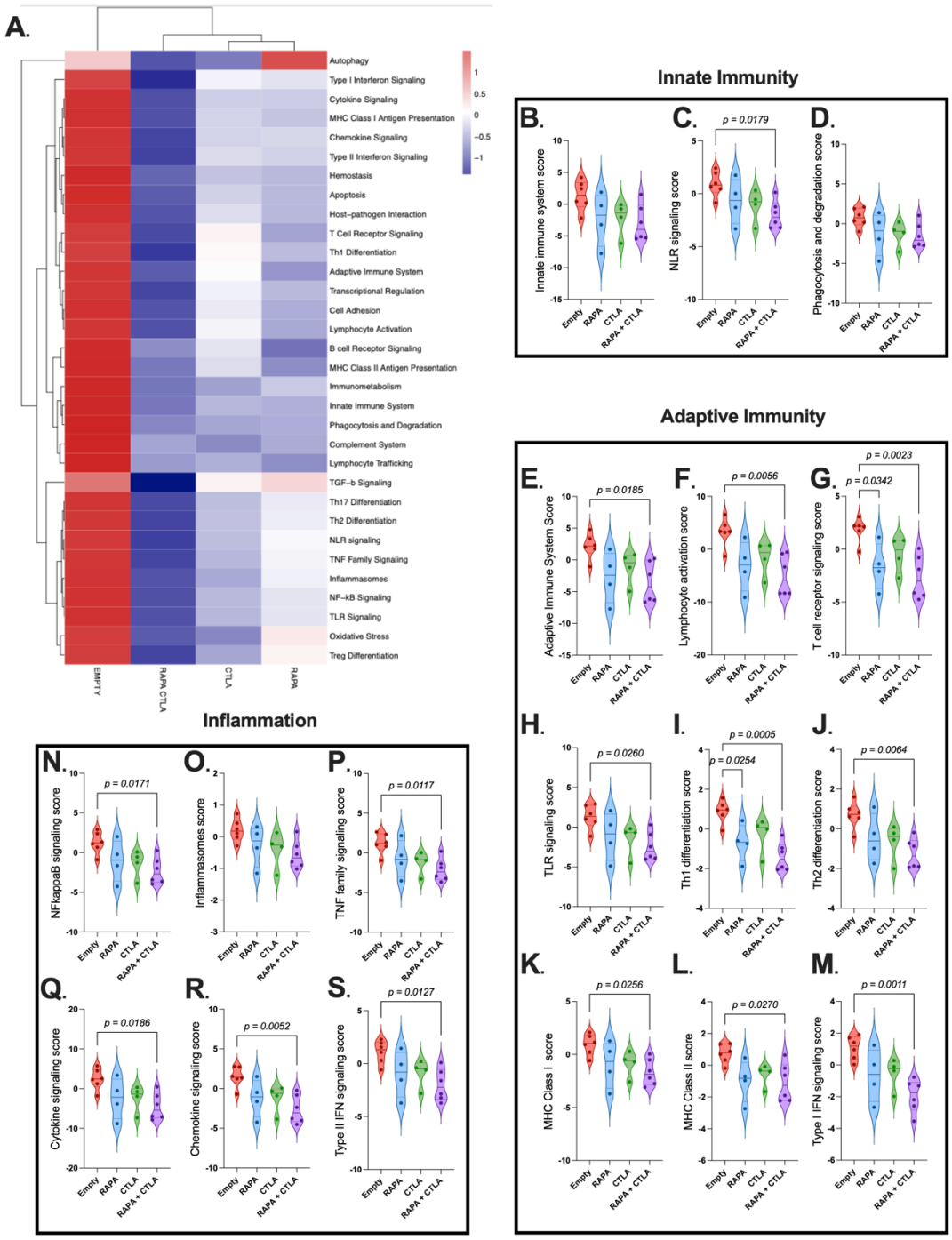


Figure 2.10: Gene set analysis scores and significance measures of key pathways involved in innate and adaptive immunity along with inflammation from murine islet allografts (BALB/c islets transplanted into diabetic C57BL/6 recipients) at 7 d post-transplant. Comparison between empty MP (EMPTY, n=6), 0.1 mg/kg rapa-MP (RAPA, n=4), CTLA-4-Ig injections (CTLA, n=4), and 0.1 mg/kg rapa-MP + CTLA-4-Ig injections (RAPA + CTLA, n=6). **A)** Heat map representation of gene set analysis pathways. **B-D)** Gene set analysis pathways involved with innate immunity. **E-M)** Gene set analysis pathways involved with enhanced adaptive immunity. **N-S)** Gene set analysis pathways that enhance inflammation.

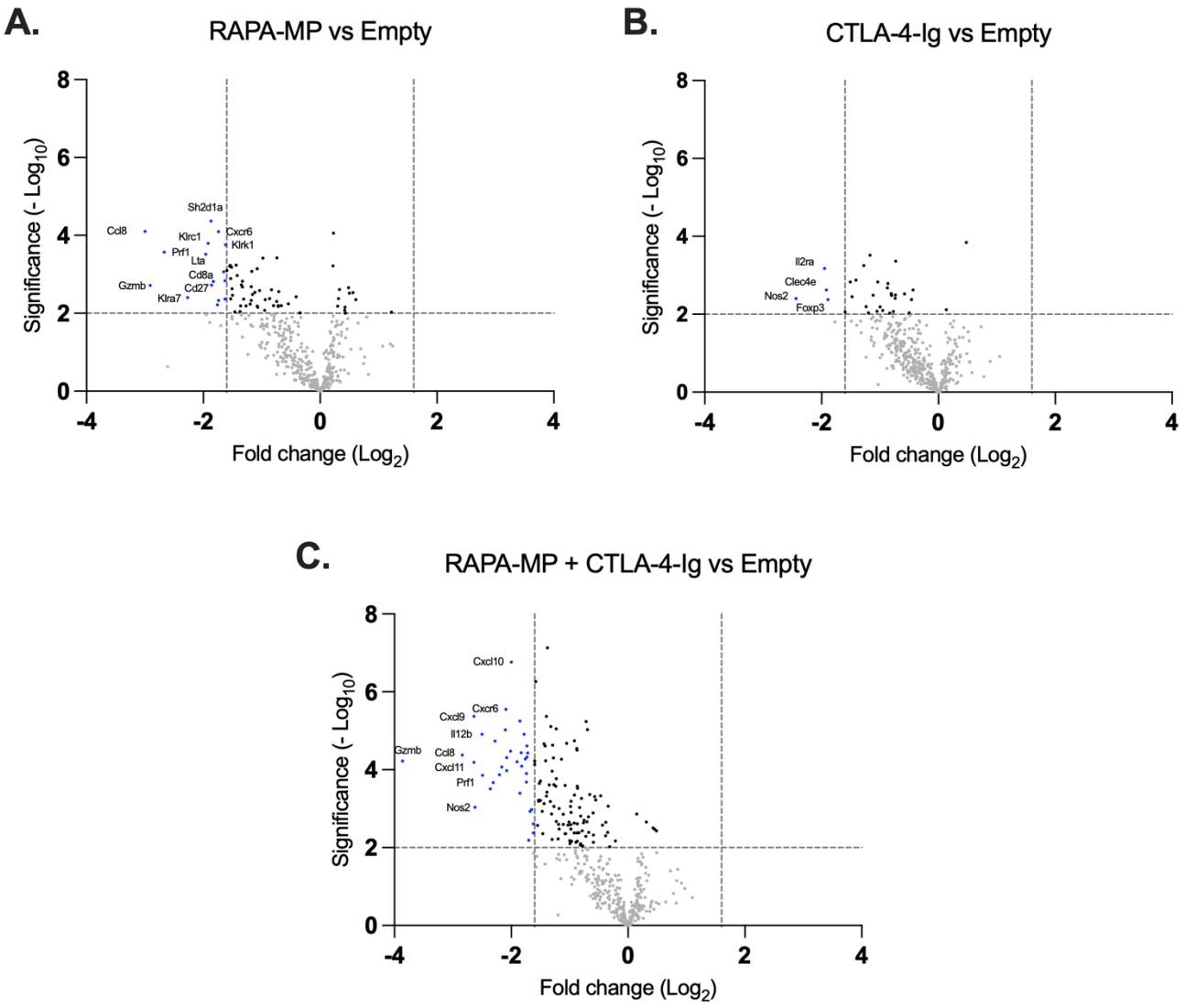


Figure 2.11: Volcano plot of fold-change in gene expression of murine islet allograft (BALB/c islets transplanted into diabetic C57BL/6 recipients) explanted at 7 d post-transplant, with empty MP + islet control recipients (n=6) serving as the reference. **A)** Gene expression fold change of 0.1 mg/kg rapa-MP (n=4) co-transplanted mice vs empty MP co-transplanted recipient controls. **B)** Gene expression fold change of CLTA-4-Ig injected + empty MP (n=4) co-transplanted mice vs empty MP co-transplanted recipient controls. **C)** Gene expression fold change of CLTA-4-Ig injected + 0.1 mg/kg rapa-MP (n=6) co-transplanted mice vs empty-MP co-transplanted recipient controls.

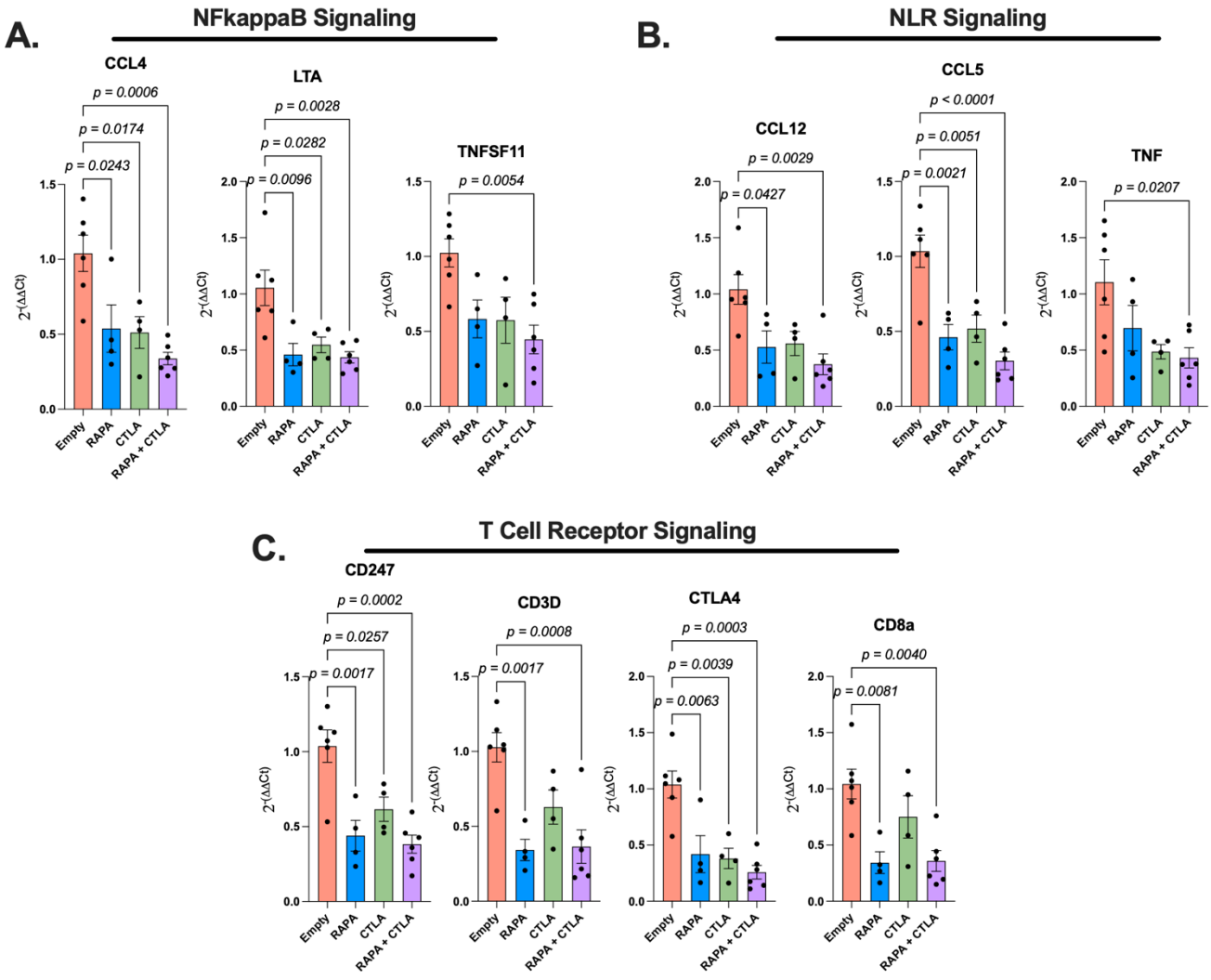


Figure 2.12: Relative gene expression involved with pathways of the adaptive and innate immunity and inflammation of murine islet allografts (BALB/c islets transplanted into diabetic C57BL/6 recipients) collected at 7 d post-transplant. **A)** Relative expression of NFkappaB pathways genes. **B)** Relative expression of NLR signaling genes. **C)** Relative expression of T cell receptor signaling genes.

2.3.6 Testing Rapa-MP in the Context of the Human Immune System – a Proof-of-Concept

Immunohistochemical analysis of human islet grafts collected at 3- and 6 w post-transplants from non-diabetic NSG-MHC I/II DKO mice (deficient in MHC I/II), injected with human PBMC at the time of ITx, allowed us to examine the role of rapa-MP in the context of the human immune system. Mice were either co-transplanted with empty MP or 0.1 mg/kg rapa-MP and grafts were stained for insulin, nuclear DNA, and CD45+ (**Figure 2.13A)**. At 3 w post-transplant, 3 of 3 empty MP grafts stained positive for CD45+ (marker for leukocytes), compared to 1 of 3 rapa-MP grafts staining positive (**Figure 2.13A)**. Automated quantification of the percentage of CD45+ cells to the graft area was comparable at 36.95 ± 2.0% and 12.22 ± 12.19% (**Figure 2.13B).** The later collection time point at 6 w yielded similar results as 4/5 empty MP grafts and 3/6 rapa-MP grafts had positive staining with comparable overall proportions of CD45+ (22.49 ± 11.81% and 20.0 ± 12.6%, respectively, **Figure 2.14C)**. These trends were also reflected in spleen samples collected at these timepoints (**Figure 2.14B,C).** Quantification of insulin-positive cells, normalized to total graft area, was compare between rapa-MP and empty MP at the respective timepoints (**Figure 2.14D).** Staining completed on human spleens for CD45+ confirmed antibody efficacy **(Supplementary Figure 4).** Quantitative results indicate a minor to no difference of rapa-MP when tested on the human immune system which may be attributed to premature graft retrieval, as insulin-positive cells were still present. Though there were a smaller fraction of grafts in the rapa-MP group that stained positive for CD45+ at both timepoints compared to empty MP controls.

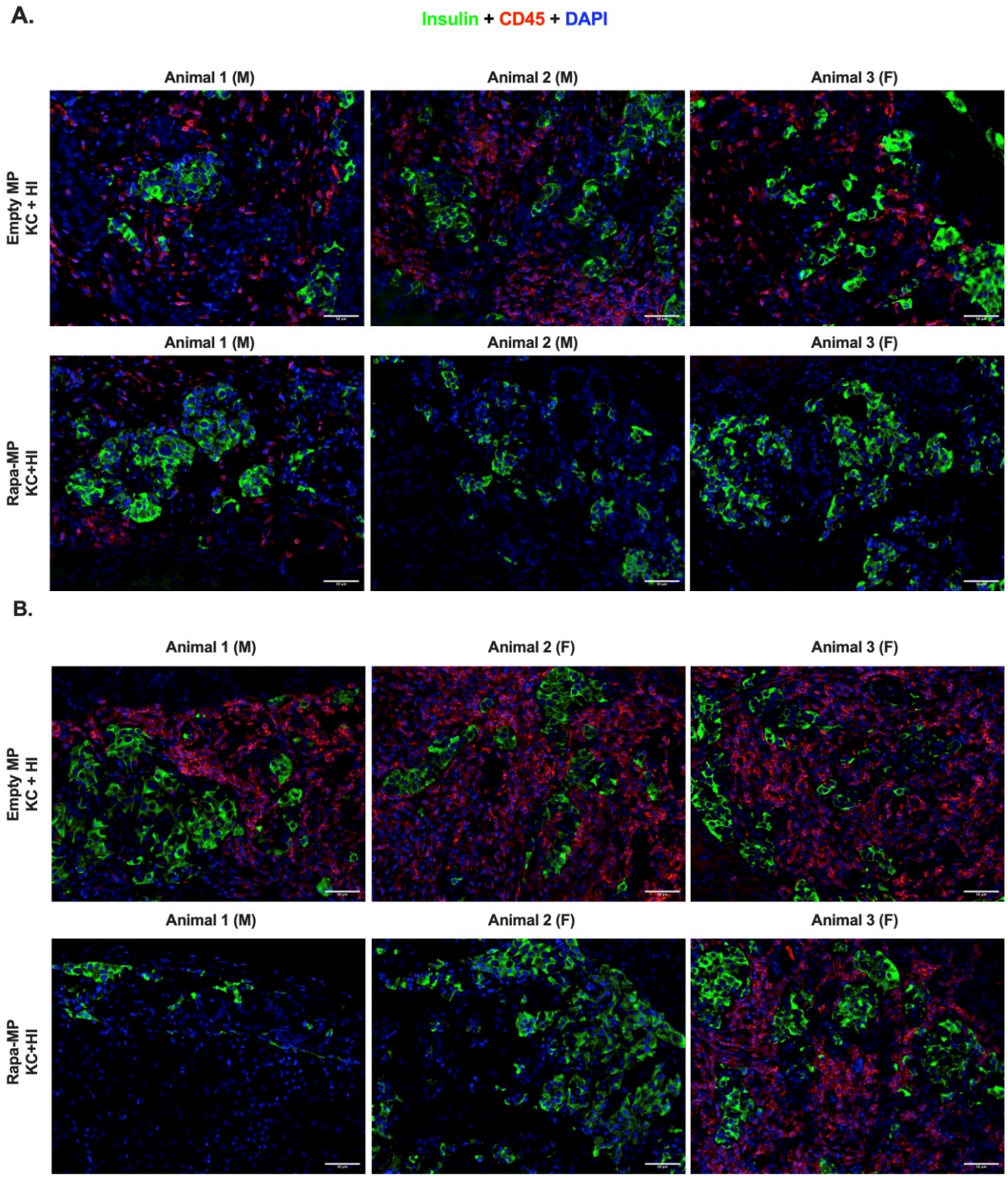


Figure 2.13: Immunohistochemistry of human islet (HI) grafts transplanted into non-diabetic NSG-MHC I/II DKO mice under the kidney capsule (KC) with empty MP or 0.1 mg/kg rapa-MP. Mice received human PBMC at the time of ITx and grafts were collected at 3 and 6 w post-transplant. Insulin positive (green), CD45+ cells (red), nuclear DNA (blue). **A)** Human islet grafts explanted at 3 w post-transplant. **B)** Human islet grafts explanted at 6 w post-transplant.

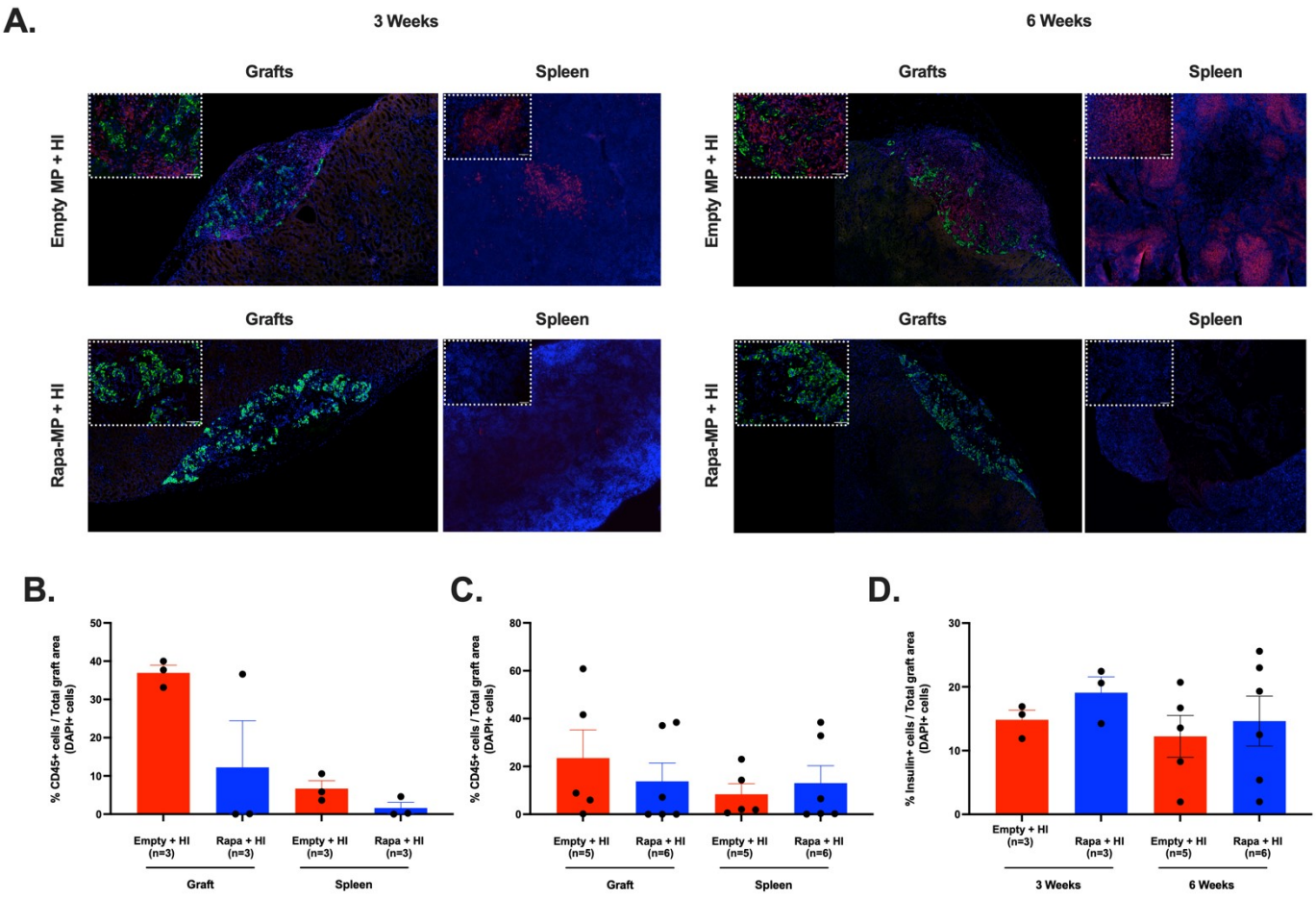


Figure 2.14: Immunohistochemistry of human islet grafts collected transplanted under the kidney capsule of NSG-MHC I/II DKO mice that were injected with human PBMC at the time of ITx. Mice were co-transplanted with either empty MP (red) or 0.1 mg/kg rapa-MP. **A)** Immunohistochemistry staining of human islet grafts and recipient's spleens for human CD45+ (red) and insulin (green) positive cells and for nuclear DNA (blue) at 3- and 6w post-transplant. **B)** Quantification of CD45+ cells at 3 w post-transplant normalized to total graft area. **C)** Quantification of CD45+ cells at 6 w post-transplant normalized to total graft area. **D)** Quantification of insulin-positive cells at 3 and 6w post-transplant normalized to total graft area.

Flowcytometric analysis of human immune cells confirmed successful engraftment in NSG-MHC I/II DKO mice that were also transplanted with human islets. At the time of ITx, recipient mice were injected i.p. with 40×10^6 PBMCs and at 3- and 6-w post-injection, spleens and peripheral blood were collected (in addition at t=0 for blood samples) for analysis. Subsequent gating of these samples indicated levels of human CD45+, CD45+CD3+, and T cell phenotype in peripheral blood and/or spleen (**Figure 2.15**). These gating strategies were used to compare the phenotypes of the engrafted human T cell population in mice that were co-transplanted with either human islets + empty MP or human islets + 0.1 mg/kg rapa-MP to ensure the treatment condition did not induce any systemic reduction in the immune cell population (**Figure 2.16**). To note, the presence of human CD3+ T cells with both CD4+ and CD8+ cells indicated the successful engraftment of NSG-MHC I/II DKO mice with human PBMCs. Moreover, CD4+FoxP3 were present, a marker for Tregs. These populations of immune cells were comparable in quantity between human islets + empty MP and human islets + 0.1 mg/kg rapa-MP transplanted groups in both spleen and peripheral blood samples at all respective time points (**Figure 2.17A,B)**. Altogether, these data indicate that injected human PBMC successfully engrafted in the ITx recipients and the rapa-MP treatment did not reduce the systemic human immune cell population or alter its phenotype compared to empty MP control recipients.

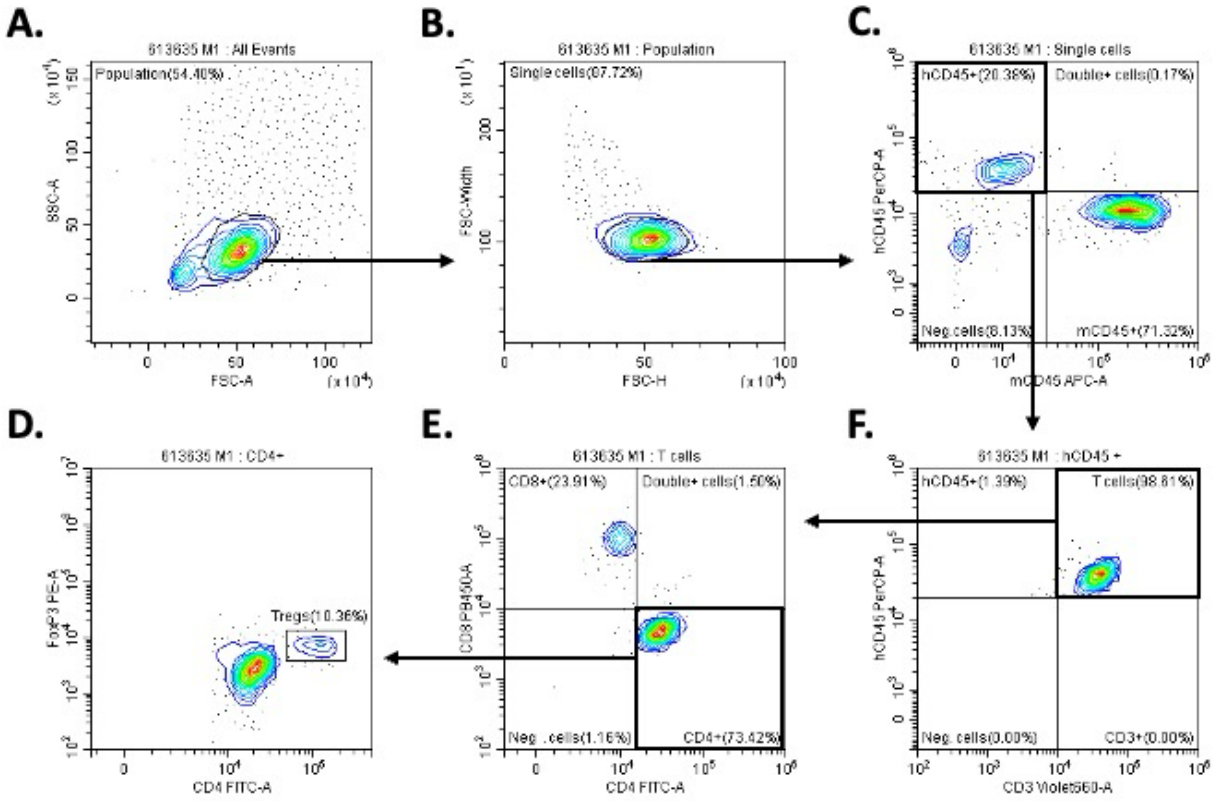


Figure 2.15: Representative flow cytometry phenotypic analysis of human T cells engrafting in NSG-MHC I/II DKO mice from peripheral blood collected at 3 w post-injection with human PBMC. Black arrows highlight the gating pathway and black boxes indicate gated sections. **A)** Cells were gated and selected based on forward versus side scatter and **B)** single cells were selected. **C)** Human CD45+ cells were gated from mouse CD45+ cells and further analyzed for **D)** CD3+ expression to determine the engrafted T cell populations. **E)** T cells were then classified as CD4+ or CD8+. **F)** Expression of FoxP3 was measured in CD4+ cell populations.

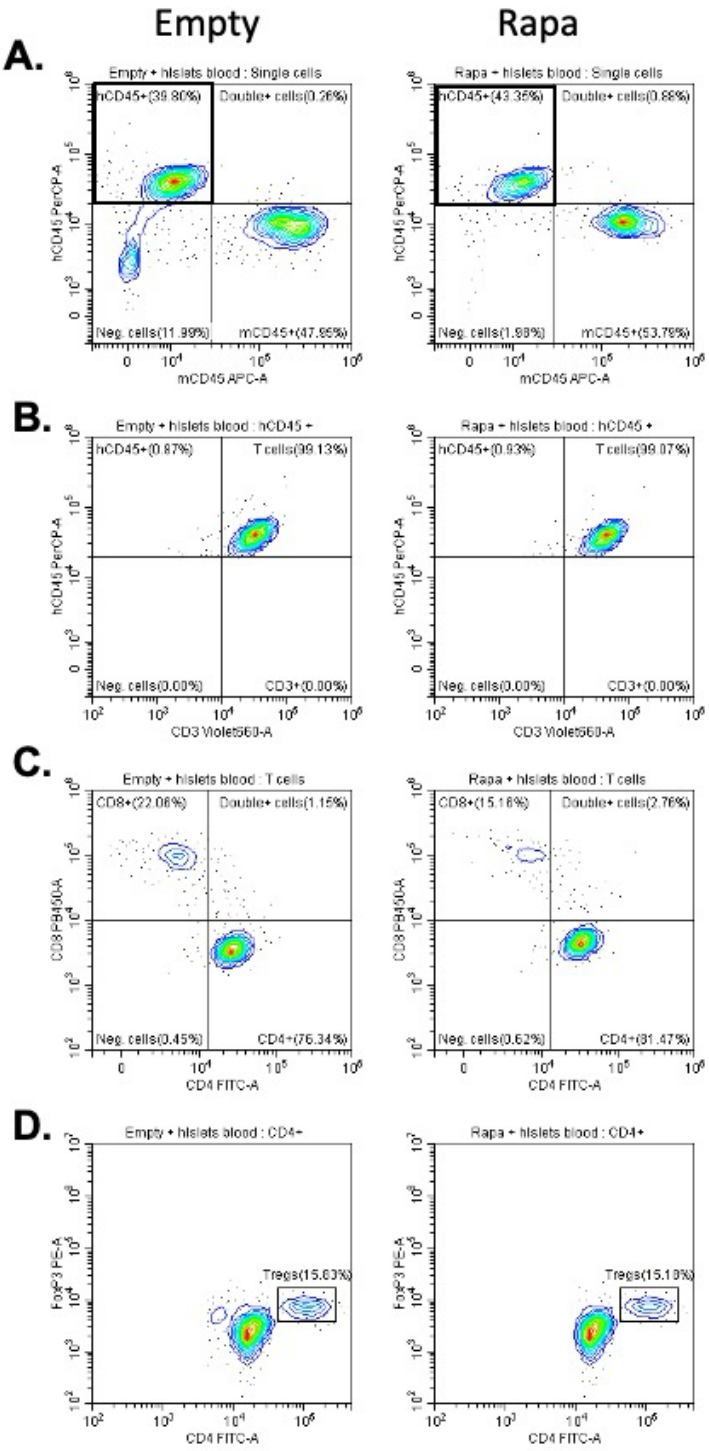


Figure 2.16: Representative flow cytometry phenotypic analysis of the engrafted T cell populations of splenocytes collected from NSG-MHC I/II DKO mice at 6 w post-injection with human PBMC. Mice were also co-transplanted with human islets + empty or rapa containing MP at the time of PMBC injection. **A)** hCD45+ cells were gated (black box) **B)** assessed for CD3+ cells. **C)** CD3+ T cells were then assessed for their expression of CD4 or CD8. **D)** Expression of FoxP3 was measured in CD4+ cells.

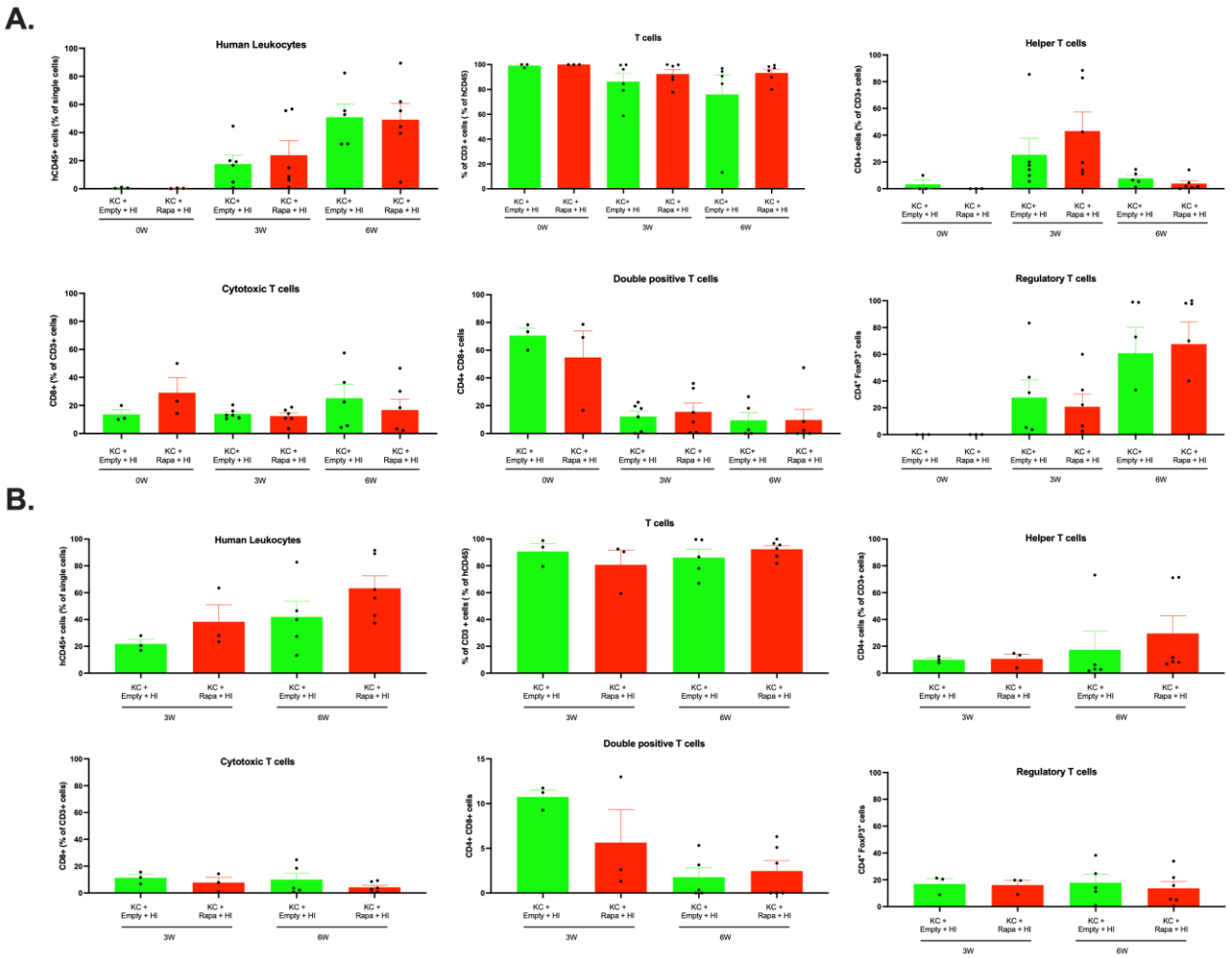


Figure 2.17: Flowcytometric quantification of human immune cells assessed in peripheral blood and single-cell splenic suspension of the engrafted T cell populations in PBMC-injected NSG-MHC I/II DKO transplanted with human islets + empty (green) or rapa (red) containing MP. **A)** Quantification of immune cells in peripheral blood collected at t=0, 3, and 6 w post-injection with PMBC. **B)** Quantification of single-cell splenic suspension collected at 3 and 6 w post-injection with PBMC.

2.4 Discussion

With the many hurdles still apparent in ITx, the procedure is limited to a select few with brittle diabetes. Concerted efforts to reduce the burden of immunosuppression, and certainly improve the long-term function of islet grafts, may broaden the application and use of this functionally curative procedure. In the present study, we successfully developed a localized drug-eluting system to reduce or abolish the requirement for toxic and chronic systemic immunosuppression. Through encapsulating rapa into MP fabricated from PLGA, we showed that these rapa-MP have sustained release and determined a non-toxic dosage with syngeneic ITx studies. Moreover, we demonstrated the profound ability of rapa-MP to delay rejection and prolong graft function when co-transplanted with allogeneic islets under the kidney capsule of diabetic mice. These effects were potentiated even further when combined with a short-course and low-dose systemic delivery of CTLA-4-Ig therapy. Thus, we highlighted the application of rapa-MP, either as a mono- or combination therapy, in promoting long-term islet allograft function.

Long-term function of murine islet allografts co-transplanted with rapa-MP may be one of the most thrilling findings of the present study. We saw that diabetic ITx recipients co-transplanted with rapa-MP not only had a delay in rejection compared to empty MP controls, but 2/6 sustained function long-term for over 200 d post-transplant. Importantly, this was the first instance that we saw the long-term survival of islet allografts when treated with our PLGA MP technology as a monotherapy. Our previous work has shown some delay in rejection as cyclosporin A loaded MP co-transplanted allografts were completely rejected by 35 d, while dexamethasone-MP monotherapy failed to prolong graft function[215,216]. Divergence in long-term outcomes may be due to the more potent immunosuppressive and tolerance induction abilities of

rapa[221]. Rapa may have also improved insulin signaling and glucose uptake, as acute treatment with rapa has been shown to enhance these pathways in myocytes and adipocytes[222]. Furthermore, the ability to synergize with other immunosuppressive agents further endorses the application of rapa in novel transplantation drug-delivery strategies[223-225]. As proposed, rapa-MP worked synergistically with a short-course and low-dose CTLA-4-Ig systemic therapy as 100% (6/6) dual therapy recipients demonstrated long-term allograft function, yet only 38% (3/8) and 33% (2/6) achieved this in the CTLA-4-Ig and rapa-MP monotherapy groups, respectively. Moreover, the dual therapy islet grafts had robust function as they displayed stronger glucose responsiveness than naïve mice, indicated by the 100 d post-transplant IPGTT. Having both systemic and localized immunosuppression may reduce the overall baseline immunogenicity and the initial 'hit' with allogenic antigens at the transplant site. Naturally, this is largely dependent on the types of immunosuppression employed and their associated mechanisms of action.

Gene expression analysis provided insights on the intragraft impact of rapa-MP and/or acute CTLA-4-Ig therapy on a cellular level. Examining pathways involved with inflammation, adaptive, and innate immunity may explain the patterns of allograft survival within each respective treatment group that was observed in the in vivo allogeneic ITx experiments. Consistent with these findings, the largest and most significant downregulation of gene expression in the presented pathways were the dual therapy grafts when compared to the empty MP control grafts. The profound significant reduction of significance score, with the majority of these pathways involved in adaptive immunity and inflammation, leaves room for speculation around the cause behind this divergence. With rapa-mediated mTORC1 inhibition, the inactivation of T and B lymphocytes through arresting their proliferation is a clear contributor. Yet only a couple of pathways of adaptive immunity were downregulated in the rapa-MP alone

grafts compared to empty MP grafts. The addition of CLTA-4-Ig can explain the more widespread downregulation of pathways involved with adaptive immunity in the rapa-MP + CTLA-4-Ig grafts. T cell activation requires two distinct signals from antigen-presenting cells, first with T cell receptor stimulation via antigen bound MHC and second with T cell-expressed costimulatory receptor binding[226]. Activation of the T cell-expressed costimulatory receptor alone yields no effect, whereas exclusive T cell receptor stimulation without costimulation induces T cell anergy[227]. As such, CTLA-4-Ig was developed to induce the latter via inhibiting costimulation of the most prominent T cell-expressed costimulatory receptor on naïve T cells, CD28. Through sharing attributes to CD28, CTLA-4-Ig binds to its ligands B7-1 (CD80) and B7-2 (CD86) with higher affinity and effectively inhibits the activation of helper and cytotoxic T cells, blocks regulatory T cell function, and induces anergy and cell death of naïve T cells[228]. These actions may explain the widespread downregulation of pathways of adaptive immunity of the dual therapy group compared to empty MP grafts. To note, the monotherapy with CTLA-4-Ig did not yield such robust differences in gene expression, and in fact no significant difference in pathways scores to empty MP grafts, which confirmed the dose and/or regimen of administration was subtherapeutic. Innate immunity pathways differed the least in all treatment groups compared to the empty MP graft control, which is consistent with the mechanisms of actions discussed. As a whole, these data corroborate allogenic ITx findings by demonstrating the synergism of combining rapa-MP with CTLA-4-Ig injections and displaying some efficacy when applying these treatments as monotherapies in downregulating genes involved with the inflammatory, adaptive, and innate immune response.

With the concept of localized drug delivery primarily promoting graft survival through modulation of the transplant site milieu, we hypothesized that tolerance induced was not

systemic in nature. Application of this principle also ensures toxicity is abolished as the low or negligible systemic drug levels should not facilitate global immune suppression and associated adverse reactions. Through the skin transplant study on long-term islet allograft recipients that received the dual therapy, we confirmed this as all donor-matched and third-party skin grafts were rejected. Yet, we observed operational tolerance generated by the dual therapy as islet allografts survived beyond the duration of third-party skin rejection despite an absence of immunosuppression at that timepoint. A significantly modest delay in donor-matched skin graft rejection was seen in the ITx recipients compared to naïve donor-matched skin grafted mice, whereas no difference in third-party skin graft survival was observed between the two groups. These findings align with our previous work, in which we ran a similar experiment with cyclosporin A loaded MP and saw an average ~10 d longer survival of donor-matched skin graft in ITx recipients to naïve skin grafted mice[215]. Prolongation of donor-matched skin allograft survival compared to naïve control skin grafted mice was also reported in mice that underwent intrahepatic ITx prior to skin grafting[229]. The apparent prolonged survival of donor-matched skin graft indicates a degree of systemic tolerance conferred by our rapa-MP + CTLA-4-Ig dual therapy approach. Mechanisms involving 'peripheral tolerance' generated towards the alloantigen in the prior ITx may be at play as by the time skin transplantation was conducted (75 d post-transplant), all the rapa should have been released by the MP and 4-5 half-lives of CTLA-4-Ig have lapsed[230]. Peripheral tolerance involves the induction of anergy, peripheral deletion, and regulation (via Treg) of alloreactive T cells[231]. Since therapeutic levels of CTLA-4-Ig or rapa at the time of skin transplant are absent, it can be assumed that antigen presentation and/or T cell activation are not impaired. Thus, it can be postulated that Treg, previously generated for allogeneic MHC-specific islet graft tolerance, attenuate the adaptive immune response to donor-

matched skin graft antigens leading to a longer survival. However, a strong immune response mounted by the abundant presentation of alloantigen by dermal dendritic cells may have tipped the balance, overcoming Treg-mediated immunoregulation. Subsequent donor-matched skin graft failure ensued indicating an islet allograft- or site-specific tolerance generated by the rapa-MP + CTLA-4-Ig approach. Though the tolerances could not withstand the adaptive immune response generated against the donor-matched skin graft antigens which led to the failure of the islet grafts thereafter. Regardless, to differentiate between the two (islet allograft- vs site-specific tolerance), transplanting donor-matched (BALB/c) islets without rapa-MP under the capsule of the contralateral kidney, after the long-term function of the primary islet graft is established, could yield a definitive conclusion. Rejection of the secondary islet allograft would indicate site-specific tolerance, whereas ongoing function may support the idea of islet allograft tolerance. Nevertheless, we demonstrated an operational tolerance generated by our dual therapy approach that we speculate as islet allograft- or site-specific.

When considering physical properties, rapa is an ideal candidate for the study as its hydrophobicity enables effective drug encapsulation with PLGA, a hydrophobic polymer. As such, a high encapsulation efficiency was seen with our rapa-MP characterization. This would be advantageous when accommodating for the transplant volume in a restrictive site. Furthermore, patterns in drug delivery may differ depending on the properties of the MP and their biodegradability. With PLGA polymers being primarily degraded via hydrolysis[232], similar and sustained degradation would be expected in vitro and in vivo. On the contrary, conflicting evidence argues for a role of enzymatic cleavage that may explain other work showing higher in vivo PLGA degradation rates[198]. Despite this mechanism and consensus being inconclusive, we speculate that it may play some role in the divergence seen with the in vitro and in vivo rapa

release experiments. However, other factors could also have contributed including human error during the in vivo rapa-MP implantation or pellet retrieval, different weights of MP being examined, and greater diffusion in vivo. Rapa release demonstrated a slower and overall lower cumulative release in vitro, compared to the in vivo experiments which showed an initial burst of rapa release that became gradual. An initial burst pattern of release may in fact be beneficial as it can attenuate host inflammatory reactions and immune cell activation pathways triggered by tissue damage from the surgery, thus improving the initial survival of the islet graft. Alternative strategies to achieve this may employ a similar strategy utilized by Fan et. al., in which they generated porous polycaprolactone (PCL) and smooth PLGA rapa-loaded MP to achieve an initial burst release with the former MP and a sustained with the latter[233]. Certainly, combining rapa-MP with other biomaterial strategies can help fine-tune drug delivery and promote the long-term function of islet grafts.

Co-localization of rapa-MP with sensitive islet cells must be done so cautiously, as the detrimental effects of rapa on beta-cell function are well established[152]. Monitoring of systemically delivered rapa can be done so through blood draws which allow the fine-tuning of dosing in patients. Contrarily, localized drug delivery is not as straightforward and there is less knowledge surrounding the pharmacokinetics of such an approach. Specifically, elucidating the pattern of distribution of drugs in the rapa-MP may prove the most challenging and vital for reducing rapa toxicity in beta-cells. These challenges stem from the inability to determine the local concentration of released rapa at any given time, which makes it hard to precisely adjust the transplanted weight of the MP to stay within a therapeutic and non-toxic range. While we transplanted our rapa-MP by targeting a daily delivered dose of rapa over 30 d based on body weight, this may have not been the most accurate method. Since rapa was not aimed to be

distributed to the entire body, a more precise dosing method may have been to target a dose based on graft volume or number of islets/IEq. Undoubtedly, this could differ between sites as the rate of diffusion could change based on the degree of site perfusion. The apparently non-toxic rapa-MP weight of 1 mg with ~350 islets was established in the murine syngeneic ITx studies and may serve as a reference for future experiments that explore alternative sites or larger animal models.

A handful of site-specific factors must be considered when applying the localized drug-eluting MP technology. First and foremost, the site must have the capacity to accommodate the larger transplant volume when islets are mixed with the MP. Next, this location must be confined in nature to prevent the migration of MP beyond the ITx site. While we showed the ability of rapa-MP + CTLA-4-Ig to confer operational tolerance, this may not always be achieved which may require occasional 'top-ups' of MP. As such, ease of redosing with MP must be accounted for. All while considering these factors, most importantly, the site must support islet grafts in restoring glycemic stability. As discussed in Chapter 1, the main site for clinical ITx is the portal vein[234]. While there are ongoing efforts to identify alternative extrahepatic sites, there has yet to be an as effective alternative[156]. For these reasons, appraising the application of our rapa-MP in the portal vein is warranted. Advantages of the portal vein include the ability to accommodate for the significant transplant volume when combining islets + MP and the proposed non-invasive method of redosing through a percutaneous catheter. A major drawback that we predict is the inability to localize these MP to the ITx site as they are much smaller than islets and thus may distribute throughout the circulation. To address this challenge, Alwahsh et. al exploited asialoglycoprotein receptor (ASGPR)-mediated endocytosis, a highly expressed receptor in hepatocytes, via binding galactose to their fibroblast growth factor-loaded PLGA particles[235].

The high affinity of ASGPR towards the PLGA-attached galactose moiety resulted in particle localization to the portal vein with improved vascularization and islet engraftment/function in mice following transplantation via the portal vein. Innovative strategies that target hepatocytes, such as the one presented, could increase rapa-MP localization to the portal vein and enable its application to this site. A promising alternative, the subcutaneous site, may also accommodate these requirements for MP application and have numerous advantages as described in Chapter 1. Application of devices, alternative cell sources, and other biomaterial approaches would be favourable in this site due to the minimal invasiveness, ease of monitoring, and large volume capacity. On that account, there are a wide array of combinations that can be examined with the MP technology.

As we observed the ability of rapa-MP to synergize with systemic therapy, combinations with novel advancements may also yield successful outcomes. Islet encapsulation has been a well-explored area of interest, with the aim of immune evasion. Certainly, rapa-MP can further serve to reduce the immunogenicity of islet allografts, as we have shown so in the present study. The profound synergistic outcome when combined with acute CTLA-4-Ig systemic administration could translate to successes with localization in islet coating. Described in Chapter 1 was Dr. Hubbart M. Tse's approach in a multilayer coating with tannic acid and poly(N-vinylpyrrolidone) (PVPON), conferring reduced immune cell infiltration, pro-inflammatory cytokine synthesis, and delayed islet allograft rejection in mice[189]. Interesting alterations to this coating would be to replace tannic acid with CTLA-4-Ig and examine the immune outcomes with/without co-transplantation of rapa-MP. Considering macroencapsulation, examining rapa-MP's role in devices could also be a promising area of study due to the confined nature and ability to control islet-to-MP distance. Devices such as the neovascularized

implantable cell homing and encapsulation (NICHE) can likely utilize our MP technology, as it is developed with ports that locally release immunosuppression[190]. Furthermore, these ports are refillable which may be of use in preserving long-term function. Additional areas for investigation include examining synergies with other immunosuppressive therapies such as a short-course of systemic daclizumab, an induction agent used in ITx[236]. Moreover, combination drug-eluting MP may be another area of interest as we have shown the success of cyclosporin A and dexamethasone-loaded MP previously[215,216]. Multi-drug-eluting MP can reduce the transplant volume compared to individually drug-loaded particles, and as such we are currently investigating their application. Lastly, the combination of rapa-MP may support the success of promising insulin-producing cell alternatives. With many of these cells being engineered to have reduced immunogenicity, as discussed in Chapter 1, co-transplantation with drug-loaded MP may further aid in addressing the immunologic barriers. Certainly, there are countless other possibilities when considering the combination of rapa-MP with other innovative strategies.

Akin to other preclinical research, there are surrounding concerns on clinical translatability. As mouse models address the shortcomings of in vitro studies and provide a cost-effective, controlled, and modifiable approach, the present study utilized these advantages to test our novel rapa-eluting MP. Of course, our rodent studies are not a perfect reflection of what may happen in humans due to distinct differences between human and mouse islets, immune systems, anatomy, and environmental exposures. To address the former two, we presented a proof-of-concept for a humanized mouse model whereby we introduced human islets and PBMCs to non-diabetic NSG-MHC I/II DKO mice**.** Although the quantification of CD45+ cell graft infiltration did not differ between empty MP and rapa-MP groups at both timepoints (3 and 6 w), we still saw a lower fraction of rapa-MP grafts staining for these leukocytes. Speculation could involve

the limited sample size, alternative methods for normalization (i.e., normalize CD45+ cells to insulin-positive cells), and a premature time chosen for graft retrieval as the insulin content was comparable between the 3 w and 6 w timepoint. Regardless, we showed the successful engraftment of human PBMCs in the NSG-MHC I/II DKO recipients which may lead to future studies examining their rejection profile in mice co-transplanted with rapa-MP in long-term studies. These NSG-MHC I/II DKO mice are exceptional for this model as they are immunodeficient and deficient in MHC class I and II expression, reducing the occurrence of graft-versus-host disease following human PBMC infusion. While this exciting approach provided a window into how rapa-MP may induce tolerance in the context of the human immune systems, there are necessary amendments to bridge the model one step closer to the clinical realm. To start, an apparent area for improvement would be to induce diabetes within these mice prior to ITx, to examine the therapeutic potential of our rapa-MP in not only delaying human islet graft rejection, but also preserving its function. Furthermore, these experiments may help provide a more accurate dosing of these rapa-MP in reference to the IEq instead of recipient weight, a concept that was previously discussed. Performing experiments with multiple different human islet donors could also be more reflective of the heterogeneity of the donor pool seen in clinical ITx. Next, examining human PBMCs that originate from a donor living with T1DM could further bridge the gap to the clinical realm. While gene expression analysis of PBMC from recent-onset T1DM patients suggested higher immune activation and lower immune regulation to healthy controls[237], we may expect a more robust ITx rejection profile if these PBMCs were employed in the mouse model. These outcomes would be more reflective of the population that typically undergoes ITx. As clinical ITx often utilizes a multi-donor infusion[238], examining the efficacy of our rapa-MP in delaying rejection in a similar fashion through performing multi-

donor islet transplantation could be another interesting study. Alternative strategies prior to clinical testing may involve scaling up with porcine or non-human primates transplant models, though this could be cost- and time-prohibitive. Taken together, these concerted efforts to reflect the clinical realm may precipitate preclinical successes into favourable clinical outcomes.

Limitations in the present study involve inherent downfalls and addressable drawbacks. As described earlier, the nature of rodent studies prevent completely accurate reflections of what may be seen clinically. Furthermore, our rodent model did not utilize the primary clinical ITx site, which can also be attributed to the difference in mouse and human anatomy along with the requirement for a confined space for the MP testing. Strides were made to bridge these limitations and work is ongoing. Minor inherent limitations also lie with our in vitro experiments examining islet viability as the 24 h duration of MP co-culturing with islets were brief due to a declining islet viability with the longer culture time. As such, these findings may not be the best indicator for in vivo toxicity in which the co-localization with MP is much longer. Addressable drawbacks involve small sample sizes in a handful of our experiments which may prevent us from making robust conclusions. Additional work staining of allografts for different immune cell markers (i.e., FoxP3, CD4+, CD8+, and CD68+ cells) may shed more light into the immune site milieu and the influence of rapa-MP. Furthermore, staining of insulin content may not be the best indicator of functional graft area as the proportion of graft staining could vary between different sections from a graft. As such, determining total insulin content of a graft can give better insights, though this would be done at the expense of examining immune cell distribution and infiltration through staining.

Overall, we proposed that localized immunomodulation with rapa-MP is an efficacious method for achieving an ‘immunosuppressive-free’ islet transplant approach. We displayed work

detailing the development, fabrication, and characterization of rapa-MP and demonstrated their ability to preserve long-term function in murine islet allografts. These long-term outcomes were potentiated when combined with low-dose and acute CTLA-4-Ig systemic therapy indicating the synergistic potential of rapa-MP. Ongoing efforts to reduce or abolish the requirement for lifelong toxic immunosuppression can certainly improve the quality-of-life and health outcomes of patients who undergo ITx. If successful, the application of ITx would be broadened as the requirement to sustain lifelong systemic immunosuppression would no longer be a criterion in patient inclusion. With the additional barriers apparent in the field, more work around identifying an alternative cell source, transplant site, and strategies that improve engraftment and survival is necessary. Despite many of these strategies being in their early stages of development including the work presented, concerted efforts in translating experimental successes into clinical settings may one day lead to a sustainable ‘functional-cure’ for diabetes in the realm of patient care.

Chapter 3: General Discussion and Summary

3.1 General Discussion

T1DM is a lifelong condition, generated by the autoimmune destruction of pancreatic beta-cells within the Islets of Langerhans. A deficit in physiologic insulin secretory abilities ensues which carries a myriad of health implications. Primary outcomes include chronic hyperglycemia when left untreated. Long-term irreversible vascular complications often follow and can range from retinopathy, nephropathy, and neuropathy to coronary artery, cerebrovascular, and peripheral vascular disease[4]. While the mainstay treatment of exogenous insulin therapy can delay many of these outcomes, this therapy is not perfect as this population still sees a significant loss in life years[7]. Moreover, exogenous insulin therapy may also induce episodes of life-threatening hypoglycemia. Advancements in automatic insulin delivery and continuous glucose monitoring address these concerns, yet they cannot recapitulate native islet function. For these reasons, islet transplantation (ITx) has been a sought-after therapy to restore physiologic glycemic regulation without the sometimes-life-threatening complications linked to exogenous insulin injections.

ITx is an established approach that can free recipients from insulin injections and reduce the occurrence of hypoglycemic episodes. Through closely mirroring the native pancreas, islet grafts essentially provide a 'functional-cure' for T1DM. Investigations as early as 1893 occurred where they transplanted fragments of sheep pancreas into a 13-year-old boy suffering from ketoacidosis[22]. However unsuccessful, efforts in the field have skyrocketed in the ~30 years since with innovations in islet isolation, immunosuppression, transplant sites, alternative cell sources, and engraftment. Despite the landmark clinical trial in 2000, *the Edmonton Protocol* in which all patients were freed from insulin injections for 1-year, major limitations are still

apparent as only a small fraction were sustained long-term[35,175]. As such efforts to address the shortcomings of the procedure are ongoing. In brief, strategies addressing the limited cell source, immediate and gradual graft attrition, and requisite lifelong systemic and toxic immunosuppression following ITx are at the forefront. Major limitations present in these areas limit the procedure to those who experience frequent and severe episodes of hypoglycemia. Recipient selection is further restricted by the ability to sustain lifelong systemic immunosuppression following transplantation. To overcome this criterion and the toxicities associated, the project explored the application of localized immunosuppression with microparticles (MP).

The central goal of this thesis was to develop a localized drug-eluting system that preserves islet graft function, effectively reducing/abolishing the need for lifelong systemic immunosuppression. We achieved this through encapsulating rapamycin (rapa), a potent immunosuppression used in clinical ITx, within biodegradable MP. The many toxicities of rapa were highlighted in Chapter 2, and along with its physical properties, made it an ideal candidate for local drug elution with PLGA. Caution was taken prior to testing rapa-MP immunomodulatory roles, as the direct toxicity of rapa on beta-cell function has been well established[152]. Therefore, in vitro OCR and syngeneic ITx studies helped us identify a non-toxic dose to transplant in the allogeneic model. The established non-toxic rapa-MP dose was transplanted in a T1DM allogeneic ITx mouse model and showed a profound long-term delay in rejection, which was synergized with acute CTLA-4-Ig systemic therapy. Our findings highlight the potential of rapa-MP as either a mono- or combination therapy in prolonging the function and survival of islet allografts. The evident synergism displayed by rapa-MP could be a fascinating area for investigation, as there is an abundance of other graft-prolonging strategies under

examination. For example, determining if there are synergies of 'pre-habilitation' exercise prior to co-transplantation with islets + rapa-MP could uncover an additional synergy (**Appendix A-4**).

We examined the tolerance generated by rapa-MP and/or acute CTLA-4-Ig therapy with skin transplant studies and intragraft gene expression analysis. Skin transplant studies demonstrated an operational tolerance generated by the rapa-MP + CTLA-4-Ig dual therapy as long-term islet graft function was ongoing throughout the third-party skin rejection. Importantly, we demonstrated an islet-graft or site-specific tolerance as all third-party and donor-matched skin grafts were completely rejected. These outcomes were as expected due to the localized nature of our rapa-MP, in which systemic immunosuppression/tolerance (i.e., donor-matched skin acceptance) should not be achieved. Additional studies that examined the intragraft gene expression gave a window into the molecular mechanisms involved with our treatments. These data corroborate the synergistic outcomes seen when combining rapa-MP + CTLA-4-Ig in our allogenic ITx model, as we observed a more widespread downregulation of genes in pathways involved with inflammatory and adaptive immunity compared to control grafts in this group. Similar trends were also observed in the rapa-MP and CTLA-4-Ig monotherapy groups, though to a lesser degree. Downregulation of genes primarily in pathways of adaptive immunity (vs innate immunity) is expected based on the mechanisms of action of rapa and CTLA-4-Ig. Certainly, more work in staining these allografts to characterize the phenotypes of immune infiltration may provide further insight into the mechanisms of rapa-MP in generating tolerance within the site milieu.

More broadly speaking, the work presented in this thesis demonstrates the promising potential of local immunosuppression, which may translate to major strides within the ITx

population. Overcoming the requisite for lifelong systemic immunosuppression would not only reduce complications associated with treatment and improve the quality-of-life of recipients, but also broaden the application of ITx to patients who cannot tolerate chronic immunosuppression. Long-term graft function may also benefit as many of the drugs used in clinical ITx are diabetogenic or toxic to beta-cell function. However, more work is required before these successes can be translated into the clinic. Although we presented the proof-of-concept humanized mouse model to test our technology in the context of the human immune system, amendments to these studies are required to more accurately reflect the clinical realm. Briefly, these include testing a diabetic human ITx model, using PBMCs from patients with T1DM, and performing multi-donor transplants. Concerted efforts to better mirror the clinical pictures, such as these, can help us make clinically favourable alterations in our MP therapy approach. Though there will likely be many more roadblocks ahead once making the transition to the clinical setting.

3.2 Summary

In this thesis, we developed and examined a localized drug-eluting MP to reduce the requirement of lifelong and toxic systemic immunosuppression. As the application of ITx is majorly hindered by an extensive immunosuppression regimen following transplant, we sought an alternative approach to reduce/abolish its requirements thereby eliminating it as a criterion in patient inclusion. To achieve this, our objective in the study was to fabricate a localized rapa-eluting MP system to subvert the immune response at the islet allograft site using the FDA-approved polymer, PLGA. Through gradual biodegradation of the rapa-MP, the drug was released locally and prolonged allograft function, and in some recipients, for greater than 200

days. Long-term success as a monotherapy was potentiated further when combined with acute CTLA-4-Ig therapy, as all allografts saw a similar trend in function. Thus, we highlight the significant role of localized immunomodulation either as a mono- or combination therapy, all without the use of chronic systemic immunosuppression. Alterations to the system and examining synergies with other therapies may one day lead to favourable outcomes in the clinical setting.

References

1. Lin X, Xu Y, Pan X, et al. Global, regional, and national burden and trend of diabetes in 195 countries and territories: an analysis from 1990 to 2025. *Scientific Reports 2020 10:1*. 2020/9// 2020;10(1):1-11. doi:10.1038/s41598-020-71908-9
2. Bilandzic A, Rosella L. The cost of diabetes in Canada over 10 years: Applying attributable health care costs to a diabetes incidence prediction model. *Health Promotion and Chronic Disease Prevention in Canada*. 2017;37(2):49-53. doi:10.24095/hpcdp.37.2.03
3. Association AD. Diagnosis and Classification of Diabetes Mellitus. *Diabetes Care*. 2010/1// 2010;33(Suppl 1):S62-S62. doi:10.2337/DC10-S062
4. Mouri M, Badireddy M. Hyperglycemia. *Observation Medicine: Principles and Protocols*. 2021/5// 2021:225-230.
5. Huang ES, Brown SES, Ewigman BG, Foley EC, Meltzer DO. Patient Perceptions of Quality of Life With Diabetes-Related Complications and Treatments. *Diabetes Care*. 2007/10// 2007;30(10):2478-2483. doi:10.2337/DC07-0499
6. Forouhi NG, Wareham NJ. Epidemiology of diabetes. *Medicine (Abingdon, England : UK Ed)*. 2014/12// 2014;42(12):698-698. doi:10.1016/J.MPMED.2014.09.007
7. Heald AH, Stedman M, Davies M, et al. Estimating life years lost to diabetes: outcomes from analysis of National Diabetes Audit and Office of National Statistics data. *Cardiovascular Endocrinology and Metabolism*. 2020:183-185. doi:10.1097/XCE.0000000000000210
8. Atkinson MA, Eisenbarth GS, Michels AW. Type 1 diabetes. *Lancet*. 2014;383(9911):69-69. doi:10.1016/S0140-6736(13)60591-7
9. T T. Type 1 and type 2 diabetes: what do they have in common? *Diabetes*. 2005/12// 2005;54 Suppl 2(SUPPL. 2)doi:10.2337/DIABETES.54.SUPPL_2.S40
10. Hypoglycemia in Diabetes: Pathophysiology, Prevalence, and Prevention - Philip Cryer - Google Books.
11. Jones J, James S, Brown F, O'Neal D, E IE. Dead in bed - A systematic review of overnight deaths in type 1 diabetes. *Diabetes Res Clin Pract*. Sep 2022;191:110042. doi:10.1016/j.diabres.2022.110042
12. Baysal N, Cameron F, Buckingham BA, Wilson DM, Bequette BW. Detecting sensor and insulin infusion set anomalies in an artificial pancreas. *Proceedings of the American Control Conference*. 2013:2929-2933. doi:10.1109/ACC.2013.6580279
13. In't Veld P, Marichal M. Microscopic anatomy of the human islet of Langerhans. *Adv Exp Med Biol*. 2010;654:1-19. doi:10.1007/978-90-481-3271-3_1
14. Brissova M, Fowler MJ, Nicholson WE, et al. Assessment of human pancreatic islet architecture and composition by laser scanning confocal microscopy. *J Histochem Cytochem*. Sep 2005;53(9):1087-97. doi:10.1369/jhc.5C6684.2005
15. Stefan Y, Orci L, Malaisse-Lagae F, Perrelet A, Patel Y, Unger RH. Quantitation of endocrine cell content in the pancreas of nondiabetic and diabetic humans. *Diabetes*. Aug 1982;31(8 Pt 1):694-700. doi:10.2337/diab.31.8.694
16. Campbell JE, Newgard CB. Mechanisms controlling pancreatic islet cell function in insulin secretion. *Nature Reviews Molecular Cell Biology*. 2021/02/01 2021;22(2):142-158. doi:10.1038/s41580-020-00317-7
17. Petersen MC, Shulman GI. Mechanisms of Insulin Action and Insulin Resistance. *Physiological Reviews*. 2018/10// 2018;98(4):2133-2223. doi:10.1152/physrev.00063.2017

18. Ahrén B. Regulation of insulin secretion by nerves and neuropeptides. *Ann Acad Med Singap*. Jan 1999;28(1):99-104.
19. Campbell Jonathan E, Drucker Daniel J. Pharmacology, Physiology, and Mechanisms of Incretin Hormone Action. *Cell Metabolism*. 2013/06/04/ 2013;17(6):819-837. doi:https://doi.org/10.1016/j.cmet.2013.04.008
20. Wachsmuth HR, Weninger SN, Duca FA. Role of the gut–brain axis in energy and glucose metabolism. *Experimental & Molecular Medicine*. 2022/04/01 2022;54(4):377-392. doi:10.1038/s12276-021-00677-w
21. Cabou C, Burcelin R. GLP-1, the gut-brain, and brain-periphery axes. *Rev Diabet Stud*. Fall 2011;8(3):418-31. doi:10.1900/rds.2011.8.418
22. Williams P. Notes on diabetes treated with extract and by grafts of sheep's pancreas. *British Medical Journal*. 1894;2:1303-4.
23. Pybus FC. NOTES ON SUPRARENAL AND PANCREATIC GRAFTING. *The Lancet*. 1924/9// 1924;204(5272):550-551. doi:10.1016/S0140-6736(01)39800-8
24. Piemonti L, Pileggi A. 25 YEARS OF THE RICORDI AUTOMATED METHOD FOR ISLET ISOLATION. *CellR4-- repair, replacement, regeneration, & reprogramming*. 2013;1(1)
25. Hellerström C. A METHOD FOR THE MICRODISSECTION OF INTACT PANCREATIC ISLETS OF MAMMALS. *European Journal of Endocrinology*. 1964/1// 1964;45(1):122-132. doi:10.1530/ACTA.0.0450122
26. Moskalewski S. Isolation and culture of the islets of Langerhans of the guinea pig. *General and Comparative Endocrinology*. 1965/6// 1965;5(3):342-353. doi:10.1016/0016-6480(65)90059-6
27. Ballinger WF, Lacy PE. Transplantation of intact pancreatic islets in rats. *Surgery*. 1972/8// 1972;72(2):175-186. doi:10.5555/URI:PII:0039606072903315
28. Kemp CB, Knight MJ, Scharp DW, Ballinger WF, Lacy PE. Effect of transplantation site on the results of pancreatic islet isografts in diabetic rats. *Diabetologia 1973 9:6*. 1973/12// 1973;9(6):486-491. doi:10.1007/BF00461694
29. Js N, De S, D B, et al. Total or near total pancreatectomy and islet autotransplantation for treatment of chronic pancreatitis. *Annals of surgery*. 1980;192(4):526-542. doi:10.1097/00000658-198010000-00011
30. Ricordi C, Lacy PE, Scharp DW. Automated Islet Isolation From Human Pancreas. *Diabetes*. 1989/1// 1989;38(Supplement 1):140-142. doi:10.2337/DIAB.38.1.S140
31. Bretzel RG, Hering BJ, Schultz AO, Geier C, Federlin K. International islet transplant registry report. *Yearbook of Cell and Tissue Transplantation 1996–1997*. 1996:153-160. doi:10.1007/978-94-009-0165-0_15
32. Shapiro AMJ, Lakey JRT, Ryan EA, et al. Islet Transplantation in Seven Patients with Type 1 Diabetes Mellitus Using a Glucocorticoid-Free Immunosuppressive Regimen. *New England Journal of Medicine*. 2000/7// 2000;343(4):230-238. doi:10.1056/nejm200007273430401
33. Tenth Annual Report. 2017;
34. Dc B, Ha K, Ph S, et al. Long-Term Follow-Up of the Edmonton Protocol of Islet Transplantation in the United States. *American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons*. 2016/2// 2016;16(2):509-517. doi:10.1111/AJT.13458

35. Marfil-Garza BA, Imes S, Verhoeff K, et al. Pancreatic islet transplantation in type 1 diabetes: 20-year experience from a single-centre cohort in Canada. *Lancet Diabetes Endocrinol*. Jul 2022;10(7):519-532. doi:10.1016/s2213-8587(22)00114-0
36. Hering BJ, Clarke WR, Bridges ND, et al. Phase 3 Trial of Transplantation of Human Islets in Type 1 Diabetes Complicated by Severe Hypoglycemia. *Diabetes Care*. Jul 2016;39(7):1230-40. doi:10.2337/dc15-1988
37. Xavier GDS. The Cells of the Islets of Langerhans. *Journal of Clinical Medicine*. 2018/3// 2018;7(3)doi:10.3390/JCM7030054
38. Briones RM, Miranda JM, Mellado-Gil JM, et al. Differential Analysis of Donor Characteristics for Pancreas and Islet Transplantation. *Transplantation Proceedings*. 2006/10// 2006;38(8):2579-2581. doi:10.1016/J.TRANSPROCEED.2006.08.040
39. Berkova Z, Saudek F, Girman P, et al. Combining Donor Characteristics with Immunohistological Data Improves the Prediction of Islet Isolation Success. *Journal of Diabetes Research*. 2016;2016doi:10.1155/2016/4214328
40. Lyon J, Manning Fox JE, Spigelman AF, et al. Research-Focused Isolation of Human Islets From Donors With and Without Diabetes at the Alberta Diabetes Institute IsletCore. *Endocrinology*. 2016/2// 2016;157(2):560-569. doi:10.1210/EN.2015-1562
41. Jr L, Gl W, Rv R, et al. Variables in organ donors that affect the recovery of human islets of Langerhans. *Transplantation*. 1996/4// 1996;61(7):1047-1053. doi:10.1097/00007890-199604150-00010
42. D OG, T K, T M, et al. The standardization of pancreatic donors for islet isolations. *Transplantation*. 2005/9// 2005;80(6):801-806. doi:10.1097/01.TP.0000172216.47547.D5
43. Lj W, T K, D OG, et al. A Multicenter Study: North American Islet Donor Score in Donor Pancreas Selection for Human Islet Isolation for Transplantation. *Cell transplantation*. 2016;25(8):1515-1523. doi:10.3727/096368916X691141
44. Gołębiewska JE, Bachul PJ, Wang L-j, et al. Validation of a New North American Islet Donor Score for Donor Pancreas Selection and Successful Islet Isolation in a Medium-Volume Islet Transplant Center. *Cell Transplantation*. 2019/2// 2019;28(2):185-185. doi:10.1177/0963689718816989
45. Saeedi P, Petersohn I, Salpea P, et al. Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas, 9th edition. *Diabetes Research and Clinical Practice*. 2019/11// 2019;157:107843-107843. doi:10.1016/J.DIABRES.2019.107843
46. Data (Charts and tables) - GODT.
47. Figliuzzi M, Cornolti R, Plati T, et al. Subcutaneous xenotransplantation of bovine pancreatic islets. *Biomaterials*. 2005/10// 2005;26(28):5640-5647. doi:10.1016/J.BIOMATERIALS.2005.02.019
48. Jr W, B P, By X, Jr L. Piscine islet xenotransplantation. *ILAR journal*. 2004;45(3):314-323. doi:10.1093/ILAR.45.3.314
49. Be T, Jc M, E S, Ms S. Production and characterization of fetal sheep pancreatic islet-like cell clusters. *Cell transplantation*. 1996;5(4):491-498. doi:10.1016/0963-6897(95)02045-4
50. O K, L J, D E, A A. Functional and morphological differentiation of fetal porcine islet-like cell clusters after transplantation into nude mice. *Diabetologia*. 1991/6// 1991;34(6):379-386. doi:10.1007/BF00403174

51. Klymiuk N, Ludwig B, Seissler J, Reichart B, Wolf E. Current Concepts of Using Pigs as a Source for Beta-Cell Replacement Therapy of Type 1 Diabetes. *Current Molecular Biology Reports 2016 2:2*. 2016/5// 2016;2(2):73-82. doi:10.1007/S40610-016-0039-1
52. Yang Y, Wang K, Wu H, et al. Genetically humanized pigs exclusively expressing human insulin are generated through custom endonuclease-mediated seamless engineering. *Journal of Molecular Cell Biology*. 2016/4// 2016;8(2):174-177. doi:10.1093/JMCB/MJW008
53. Gs K, Jf E, Z A, M F, Gl W, Rv R. Microencapsulation of neonatal porcine islets: long-term reversal of diabetes in nude mice and in vitro protection from human complement mediated cytolysis. *Transplantation proceedings*. 1997;29(4):2128-2128. doi:10.1016/S0041-1345(97)00259-5
54. K C, Gs K, Z M, et al. Long-term survival of neonatal porcine islets in nonhuman primates by targeting costimulation pathways. *Nature medicine*. 2006/3// 2006;12(3):304-306. doi:10.1038/NM1375
55. Bj H, M W, Ml G, et al. Prolonged diabetes reversal after intraportal xenotransplantation of wild-type porcine islets in immunosuppressed nonhuman primates. *Nature medicine*. 2006/3// 2006;12(3):301-303. doi:10.1038/NM1369
56. Cg G, O K, A T, et al. Transplantation of porcine fetal pancreas to diabetic patients. *Lancet (London, England)*. 1994/11// 1994;344(8934):1402-1404. doi:10.1016/S0140-6736(94)90570-3
57. S M, P T, J B, et al. Clinical porcine islet xenotransplantation under comprehensive regulation. *Transplantation proceedings*. 2014;46(6):1992-1995. doi:10.1016/J.TRANSPROCEED.2014.06.008
58. S M, A A, C W, S W, Rb E. Clinical Benefit of Islet Xenotransplantation for the Treatment of Type 1 Diabetes. *EBioMedicine*. 2016/10// 2016;12:255-262. doi:10.1016/J.EBIOM.2016.08.034
59. Griffith BP, Goerlich CE, Singh AK, et al. Genetically Modified Porcine-to-Human Cardiac Xenotransplantation. *N Engl J Med*. Jul 7 2022;387(1):35-44. doi:10.1056/NEJMoa2201422
60. First gene-edited pig kidney transplant. *Nat Biotechnol*. Apr 2024;42(4):543. doi:10.1038/s41587-024-02223-1
61. Assady S, Maor G, Amit M, Itskovitz-Eldor J, Skorecki KL, Tzukerman M. Insulin Production by Human Embryonic Stem Cells. *Diabetes*. 2001/8// 2001;50(8):1691-1697. doi:10.2337/DIABETES.50.8.1691
62. Segev H, Fishman B, Ziskind A, Shulman M, Itskovitz-Eldor J. Differentiation of Human Embryonic Stem Cells into Insulin-Producing Clusters. *STEM CELLS*. 2004/5// 2004;22(3):265-274. doi:10.1634/STEMCELLS.22-3-265
63. D'Amour KA, Bang AG, Eliazer S, et al. Production of pancreatic hormone–expressing endocrine cells from human embryonic stem cells. *Nature Biotechnology 2006 24:11*. 2006/10// 2006;24(11):1392-1401. doi:10.1038/nbt1259
64. Kroon E, Martinson LA, Kadoya K, et al. Pancreatic endoderm derived from human embryonic stem cells generates glucose-responsive insulin-secreting cells in vivo. *Nature Biotechnology 2008 26:4*. 2008/2// 2008;26(4):443-452. doi:10.1038/nbt1393
65. Shapiro AMJ, Thompson D, Donner TW, et al. Insulin expression and C-peptide in type 1 diabetes subjects implanted with stem cell-derived pancreatic endoderm cells in an encapsulation device. *Cell reports Medicine*. 2021/12// 2021;2(12)doi:10.1016/J.XCRM.2021.100466

66. Ramzy A, Thompson DM, Ward-Hartstonge KA, et al. Implanted pluripotent stem-cell-derived pancreatic endoderm cells secrete glucose-responsive C-peptide in patients with type 1 diabetes. *Cell stem cell*. 2021/12// 2021;28(12):2047-2061.e5. doi:10.1016/J.STEM.2021.10.003
67. Keymeulen B, De Groot K, Jacobs-Tulleneers-Thevissen D, et al. Encapsulated stem cell-derived β cells exert glucose control in patients with type 1 diabetes. *Nat Biotechnol*. Nov 27 2023;doi:10.1038/s41587-023-02055-5
68. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell*. 2006/8// 2006;126(4):663-676. doi:10.1016/J.CELL.2006.07.024
69. Zhang D, Jiang W, Liu M, et al. Highly efficient differentiation of human ES cells and iPS cells into mature pancreatic insulin-producing cells. *Cell Research 2009 19:4*. 2009/3// 2009;19(4):429-438. doi:10.1038/cr.2009.28
70. Nostro MC, Keller G. Generation of beta cells from human pluripotent stem cells: Potential for regenerative medicine. *Seminars in Cell & Developmental Biology*. 2012/8// 2012;23(6):701-710. doi:10.1016/J.SEMCDB.2012.06.010
71. Kondo Y, Toyoda T, Inagaki N, Osafune K. iPSC technology-based regenerative therapy for diabetes. *Journal of diabetes investigation*. 2018/3// 2018;9(2):234-243. doi:10.1111/JDI.12702
72. Wu J, Li T, Guo M, et al. Treating a type 2 diabetic patient with impaired pancreatic islet function by personalized endoderm stem cell-derived islet tissue. *Cell Discovery*. 2024/04/30 2024;10(1):45. doi:10.1038/s41421-024-00662-3
73. REICHMAN TW, RICORDI C, NAJI A, et al. 836-P: Glucose-Dependent Insulin Production and Insulin-Independence in Type 1 Diabetes from Stem Cell–Derived, Fully Differentiated Islet Cells—Updated Data from the VX-880 Clinical Trial. *Diabetes*. 2023;72(Supplement_1)doi:10.2337/db23-836-P
74. Dr M, Jp H, As R, Ba N, Df T, Rc T. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia*. 1985/7// 1985;28(7):412-419. doi:10.1007/BF00280883
75. Takita M, Matsumoto S, Qin H, et al. Secretory unit of islet transplant objects (SUITO) index can predict severity of hypoglycemic episodes in clinical islet cell transplantation. *Cell Transplantation*. 2012;21(1):91-98. doi:10.3727/096368911X580617
76. Caumo A, Maffi P, Nano R, et al. Transplant Estimated Function. *Diabetes Care*. 2008/2// 2008;31(2):301-305. doi:10.2337/DC07-0975
77. Forbes S, Oram RA, Smith A, et al. Validation of the BETA-2 Score: An Improved Tool to Estimate Beta Cell Function After Clinical Islet Transplantation Using a Single Fasting Blood Sample. *American Journal of Transplantation*. 2016/9// 2016;16(9):2704-2704. doi:10.1111/AJT.13807
78. Richards TM, Sun A, Hayat H, et al. Current Progress and Perspective: Clinical Imaging of Islet Transplantation. *Life*. 2020/9// 2020;10(9):1-14. doi:10.3390/LIFE10090213
79. Senior PA, Kin T, Shapiro J, Koh A. Islet Transplantation at the University of Alberta: Status Update and Review of Progress over the Last Decade. *Canadian Journal of Diabetes*. 2012/2// 2012;36(1):32-37. doi:10.1016/J.JCJD.2012.01.002
80. C T, H Z, P M, et al. Positron-emission tomography imaging of early events after transplantation of islets of Langerhans. *Transplantation*. 2005/2// 2005;79(3):353-355. doi:10.1097/01.TP.0000149501.50870.9D

81. T E, O E, A S, et al. Positron emission tomography: a real-time tool to quantify early islet engraftment in a preclinical large animal model. *Transplantation*. 2007/10// 2007;84(7):893-898. doi:10.1097/01.TP.0000284730.86567.9F
82. Bennet W, Sundberg B, Groth CG, et al. Incompatibility between human blood and isolated islets of Langerhans: a finding with implications for clinical intraportal islet transplantation? *Diabetes*. 1999/10// 1999;48(10):1907-1914. doi:10.2337/DIABETES.48.10.1907
83. W B, Cg G, R L, B N, O K. Isolated human islets trigger an instant blood mediated inflammatory reaction: implications for intraportal islet transplantation as a treatment for patients with type 1 diabetes. *Upsala journal of medical sciences*. 2000;105(2):125-133. doi:10.1517/03009734000000059
84. L M, H J, A L, et al. Production of tissue factor by pancreatic islet cells as a trigger of detrimental thrombotic reactions in clinical islet transplantation. *Lancet (London, England)*. 2002/12// 2002;360(9350):2039-2045. doi:10.1016/S0140-6736(02)12020-4
85. M H, S L, A K-P, B N, O K. Characterization of Innate Immunity in an Extended Whole Blood Model of Human Islet Allotransplantation. *Cell transplantation*. 2016;25(3):503-515. doi:10.3727/096368915X688461
86. U J, G E, B N, O K. Composite islet-endothelial cell grafts: a novel approach to counteract innate immunity in islet transplantation. *American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons*. 2005/11// 2005;5(11):2632-2639. doi:10.1111/J.1600-6143.2005.01076.X
87. Cabric S, Sanchez J, Lundgren T, et al. Islet Surface Heparinization Prevents the Instant Blood-Mediated Inflammatory Reaction in Islet Transplantation. *Diabetes*. 2007/8// 2007;56(8):2008-2015. doi:10.2337/DB07-0358
88. Johansson H, Goto M, Siegbahn A, Elgue G, Korsgren O, Nilsson B. Low Molecular Weight Dextran Sulfate: A Strong Candidate Drug to Block IBMIR in Clinical Islet Transplantation. *American Journal of Transplantation*. 2006/2// 2006;6(2):305-312. doi:10.1111/J.1600-6143.2005.01186.X
89. L O, Kn E, G E, R L, O K, B N. Inhibition of thrombin abrogates the instant blood-mediated inflammatory reaction triggered by isolated human islets: possible application of the thrombin inhibitor melagatran in clinical islet transplantation. *Diabetes*. 2002;51(6):1779-1784. doi:10.2337/DIABETES.51.6.1779
90. A K, P S, A S, et al. Insulin-heparin infusions peritransplant substantially improve single-donor clinical islet transplant success. *Transplantation*. 2010/2// 2010;89(4):465-471. doi:10.1097/TP.0B013E3181C478FD
91. Delaune V, Berney T, Lacotte S, Toso C. Intraportal islet transplantation: the impact of the liver microenvironment. *Transplant International*. 2017/3// 2017;30(3):227-238. doi:10.1111/TRI.12919
92. Po C, F P, A A, P L. Markedly decreased oxygen tension in transplanted rat pancreatic islets irrespective of the implantation site. *Diabetes*. 2001;50(3):489-495. doi:10.2337/DIABETES.50.3.489
93. M G, W M, E B, et al. Central necrosis in isolated hypoxic human pancreatic islets: evidence for postisolation ischemia. *Cell transplantation*. 2005;14(1):67-76. doi:10.3727/000000005783983287

94. Md M, S J, P W, G F, F H, K M. Angiogenesis and hemodynamics of microvasculature of transplanted islets of Langerhans. *Diabetes*. 1989;38 Suppl 1(SUPPL. 1):199-201. doi:10.2337/DIAB.38.1.S199
95. Vajkoczy P, Olofsson AM, Lehr HA, et al. Histogenesis and ultrastructure of pancreatic islet graft microvasculature. Evidence for graft revascularization by endothelial cells of host origin. *The American Journal of Pathology*. 1995;146(6):1397-1397.
96. R O, J O, U P, Po C. Increased numbers of low-oxygenated pancreatic islets after intraportal islet transplantation. *Diabetes*. 2011/9// 2011;60(9):2350-2353. doi:10.2337/DB09-0490
97. Scharp DW, Lacy PE, Santiago JV, et al. Insulin independence after islet transplantation into type I diabetic patient. *Diabetes*. 1990;39(4):515-518. doi:10.2337/DIAB.39.4.515
98. Zheng X, Zheng X, Wang X, et al. Acute hypoxia induces apoptosis of pancreatic β-cell by activation of the unfolded protein response and upregulation of CHOP. *Cell Death & Disease 2012 3:6*. 2012/6// 2012;3(6):e322-e322. doi:10.1038/cddis.2012.66
99. Paraskevas S, Maysinger D, Wang R, Duguid WP, Rosenberg L. Cell loss in isolated human islets occurs by apoptosis. *Pancreas*. 2000/4// 2000;20(3):270-276. doi:10.1097/00006676-200004000-00008
100. Davalli AM, Ogawa Y, Ricordi C, Scharp DW, Bonner-Weir S, Weir GC. A selective decrease in the beta cell mass of human islets transplanted into diabetic nude mice. *Transplantation*. 1995/3// 1995;59(6):817-820. doi:10.1097/00007890-199503000-00003
101. Carlsson PO, Palm F, Andersson A, Liss P. Markedly Decreased Oxygen Tension in Transplanted Rat Pancreatic Islets Irrespective of the Implantation Site. *Diabetes*. 2001/3// 2001;50(3):489-495. doi:10.2337/DIABETES.50.3.489
102. Nielsen JH, Mandrup-Poulsen T, Nerup J. Direct effects of cyclosporin A on human pancreatic beta-cells. *Diabetes*. 1986;35(9):1049-1052. doi:10.2337/DIAB.35.9.1049
103. Laube F, Blech W, Schröder D, Besch W, Hildebrandt W, Hahn HJ. The Action of Cyclosporin A on the Endocrine Pancreas in Vitro-Functional and Morphological Studies. *Experimental and Clinical Endocrinology and Diabetes*. 1986;87(1):69-78. doi:10.1055/s-0029-1210525
104. Hahn HJ, Dunger A, Laube F, et al. Reversibility of the acute toxic effect of cyclosporin A on pancreatic B cells of Wistar rats. *Diabetologia*. 1986/8// 1986;29(8):489-494. doi:10.1007/BF00453499
105. Nerup J, Platz P, Andersen OO, et al. HL-A antigens and diabetes mellitus. *Lancet (London, England)*. 1974/10// 1974;2(7885):864-866. doi:10.1016/S0140-6736(74)91201-X
106. Choo SY. The HLA System: Genetics, Immunology, Clinical Testing, and Clinical Implications. *Yonsei Medical Journal*. 2007/2// 2007;48(1):11-11. doi:10.3349/YMJ.2007.48.1.11
107. Van Lummel M, Van Veelen PA, De Ru AH, et al. Discovery of a Selective Islet Peptidome Presented by the Highest-Risk HLA-DQ8trans Molecule. *Diabetes*. 2016/3// 2016;65(3):732-741. doi:10.2337/DB15-1031
108. Noble JA, Valdes AM. Genetics of the HLA Region in the Prediction of Type 1 Diabetes. *Current diabetes reports*. 2011/12// 2011;11(6):533-533. doi:10.1007/S11892-011-0223-X
109. Noble JA, Valdes AM, Varney MD, et al. HLA Class I and Genetic Susceptibility to Type 1 Diabetes: Results From the Type 1 Diabetes Genetics Consortium. *Diabetes*. 2010/11// 2010;59(11):2972-2972. doi:10.2337/DB10-0699

110. Han S, Donelan W, Wang H, Reeves W, Yang LJ. Novel autoantigens in type 1 diabetes. *American Journal of Translational Research*. 2013;5(4):379-379.
111. Stadinski B, Kappler J, Eisenbarth GS. Molecular targeting of islet autoantigens. *Immunity*. 2010/4// 2010;32(4):446-456. doi:10.1016/J.IMMUNI.2010.04.008
112. Krogvold L, Wiberg A, Edwin B, et al. Insulitis and characterisation of infiltrating T cells in surgical pancreatic tail resections from patients at onset of type 1 diabetes. *Diabetologia*. 2016/3// 2016;59(3):492-501. doi:10.1007/S00125-015-3820-4
113. Anderson AM, Landry LG, Alkanani AA, et al. Human islet T cells are highly reactive to preproinsulin in type 1 diabetes. *Proceedings of the National Academy of Sciences of the United States of America*. 2021/10// 2021;118(41)doi:10.1073/PNAS.2107208118/-/DCSUPPLEMENTAL
114. Bresson D, Von Herrath M. Immunotherapy for the Prevention and Treatment of Type 1 Diabetes. *Diabetes Care*. 2009/10// 2009;32(10):1753-1768. doi:10.2337/DC09-0373
115. Makhlouf L, Kishimoto K, Smith RN, et al. The role of autoimmunity in islet allograft destruction: major histocompatibility complex class II matching is necessary for autoimmune destruction of allogeneic islet transplants after T-cell costimulatory blockade. *Diabetes*. 2002/11// 2002;51(11):3202-3210. doi:10.2337/DIABETES.51.11.3202
116. Woehrle M, Markmann J, Silvers W, Barker C, Naji A. Transplantation of cultured pancreatic islets to BB rats. *undefined*. 1986;
117. Markmann JF, Posselt AM, Bassiri H, et al. Major-histocompatibility-complex restricted and nonrestricted autoimmune effector mechanisms in BB rats. *Transplantation*. 1991;52(4):662-667. doi:10.1097/00007890-199110000-00016
118. Huurman VAL, Hilbrands R, Pinkse GGM, et al. Cellular Islet Autoimmunity Associates with Clinical Outcome of Islet Cell Transplantation. *PLoS ONE*. 2008/6// 2008;3(6)doi:10.1371/JOURNAL.PONE.0002435
119. Monti P, Scirpoli M, Maffi P, et al. Islet transplantation in patients with autoimmune diabetes induces homeostatic cytokines that expand autoreactive memory T cells. *The Journal of Clinical Investigation*. 2008/5// 2008;118(5):1806-1806. doi:10.1172/JCI35197
120. Marino J, Paster J, Benichou G. Allorecognition by T lymphocytes and allograft rejection. *Frontiers in Immunology*. 2016;7(DEC):582-582. doi:10.3389/FIMMU.2016.00582/BIBTEX
121. Hu M, Wu J, Zhang GY, et al. Selective depletion of alloreactive T cells leads to long-term islet allograft survival across a major histocompatibility complex mismatch in diabetic mice. *Cell Transplantation*. 2013/10// 2013;22(10):1929-1941. doi:10.3727/096368912X658025
122. Bellin MD, Barton FB, Heitman A, et al. Potent Induction Immunotherapy Promotes Long-Term Insulin Independence After Islet Transplantation in Type 1 Diabetes. *American Journal of Transplantation*. 2012/6// 2012;12(6):1576-1583. doi:10.1111/J.1600-6143.2011.03977.X
123. Bellin MD, Kandaswamy R, Parkey J, et al. Prolonged insulin independence after islet allotransplants in recipients with type 1 diabetes. *American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons*. 2008/11// 2008;8(11):2463-2470. doi:10.1111/J.1600-6143.2008.02404.X
124. Hering BJ, Kandaswamy R, Ansite JD, et al. Single-donor, marginal-dose islet transplantation in patients with type 1 diabetes. *JAMA*. 2005/2// 2005;293(7):830-835. doi:10.1001/JAMA.293.7.830

125. Maffi P, Lundgren T, Tufveson G, et al. Targeting CXCR1/2 does not improve insulin secretion after pancreatic islet transplantation: A phase 3, double-blind, randomized, placebo-controlled trial in type 1 diabetes. *Diabetes Care*. 2020/4// 2020;43(4):710-718. doi:10.2337/DC19-1480/-/DC1
126. Am S, Jr L, Ea R, et al. Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. *The New England journal of medicine*. 2000/7// 2000;343(4):230-238. doi:10.1056/NEJM200007273430401
127. Malek TR, Castro I. Interleukin-2 Receptor Signaling: At the Interface between Tolerance and Immunity. *Immunity*. 2010/8// 2010;33(2):153-153. doi:10.1016/J.IMMUNI.2010.08.004
128. Kapic E, Becic F, Kusturica J. Basiliximab, mechanism of action and pharmacological properties. *Medicinski arhiv*. 2004;58(6):373-376.
129. Sun ZJ, Du X, Su LL, Zhang XD, Wang W. Efficacy and Safety of Basiliximab Versus Daclizumab in Kidney Transplantation: A Meta-Analysis. *Transplantation proceedings*. 2015/10// 2015;47(8):2439-2445. doi:10.1016/J.TRANSPROCEED.2015.08.009
130. Genestier L, Fournel S, Flacher M, Assossou O, Revillard J-P, Bonnefoy-Berard N. Induction of Fas (Apo-1, CD95)-Mediated Apoptosis of Activated Lymphocytes by Polyclonal Antithymocyte Globulins. *Blood*. 1998/4// 1998;91(7):2360-2368. doi:10.1182/BLOOD.V91.7.2360
131. Starzl TE, Marchioro TL, Hutchison DE, Porter KA, Cerilli GJ, Brettschneider L. The clinical use of antilymphocyte globulin in renal homotransplantation. *Transplantation*. 1967;5(4):1100-1105. doi:10.1097/00007890-196707001-00045
132. Gaber OO, First MR, Tesi RJ, et al. Results of the double-blind, randomized, multicenter, phase III clinical trial of Thymoglobulin versus Atgam in the treatment of acute graft rejection episodes after renal transplantation. *Transplantation*. 1998/7// 1998;66(1):29-37. doi:10.1097/00007890-199807150-00005
133. Mancusi A, Piccinelli S, Velardi A, Pierini A. The effect of TNF-α on regulatory T cell function in graft-versus-host disease. *Frontiers in Immunology*. 2018/2// 2018;9(FEB):356-356. doi:10.3389/FIMMU.2018.00356/BIBTEX
134. Brandhorst D, Brandhorst H, Acreman S, Abraham A, Johnson PRV. High Concentrations of Etanercept Reduce Human Islet Function and Integrity. *Journal of Inflammation Research*. 2021/2// 2021;14:599-610. doi:10.2147/JIR.S294663
135. Rowan WC, Hale G, Tite JP, Brett SJ. Cross-linking of the CAMPATH-1 antigen (CD52) triggers activation of normal human T lymphocytes. *International immunology*. 1995/1// 1995;7(1):69-77. doi:10.1093/INTIMM/7.1.69
136. Watanabe T, Masuyama Ji, Sohma Y, et al. CD52 is a novel costimulatory molecule for induction of CD4+ regulatory T cells. *Clinical immunology (Orlando, Fla)*. 2006/9// 2006;120(3):247-259. doi:10.1016/J.CLIM.2006.05.006
137. Baskar S, Klein AL, Zeft A. The Use of IL-1 Receptor Antagonist (Anakinra) in Idiopathic Recurrent Pericarditis: A Narrative Review. *Cardiology Research and Practice*. 2016;2016doi:10.1155/2016/7840724
138. M K, Wj L, Jm D, et al. Cyclosporin A inhibits T-cell growth factor gene expression at the level of mRNA transcription. *Proceedings of the National Academy of Sciences of the United States of America*. 1984;81(16):5214-5218. doi:10.1073/PNAS.81.16.5214
139. Herold KC, Lancki DW, Moldwin RL, Fitch FW. Immunosuppressive effects of cyclosporin A. 1986;

140. Matsuda S, Koyasu S. Mechanisms of action of cyclosporine. *Immunopharmacology*. 2000/5// 2000;47(2-3):119-125. doi:10.1016/S0162-3109(00)00192-2
141. Hermann-Kleiter N, Baier G. NFAT pulls the strings during CD4+ T helper cell effector functions. *Blood*. 2010/4// 2010;115(15):2989-2997. doi:10.1182/BLOOD-2009-10-233585
142. Thomson AW, Bonham CA, Zeevi A. Mode of action of tacrolimus (FK506): molecular and cellular mechanisms. *Therapeutic drug monitoring*. 1995;17(6):584-591. doi:10.1097/00007691-199512000-00007
143. Almawi WY, Melemedjian OK. Clinical and mechanistic differences between FK506 (tacrolimus) and cyclosporin A. *Nephrology Dialysis Transplantation*. 2000/12// 2000;15(12):1916-1918. doi:10.1093/NDT/15.12.1916
144. C V, A K, Sn S. Rapamycin (AY-22,989), a new antifungal antibiotic. I. Taxonomy of the producing streptomycete and isolation of the active principle. *The Journal of antibiotics*. 1975;28(10):721-726. doi:10.7164/ANTIBIOTICS.28.721
145. M J-U, Jl G, J N, M T, Ze T, R M. Distinct signaling mechanisms of mTORC1 and mTORC2 in glioblastoma multiforme: a tale of two complexes. *Advances in biological regulation*. 2015/1// 2015;57:64-74. doi:10.1016/J.JBIOR.2014.09.004
146. Thomson AW, Turnquist HR, Raimondi G. Immunoregulatory Functions of mTOR Inhibition. *Nature reviews Immunology*. 2009/5// 2009;9(5):324-324. doi:10.1038/NRI2546
147. H L, W D, H C, M M, J W. The effect of rapamycin on T cell development in mice. *European journal of immunology*. 1994;24(3):692-701. doi:10.1002/EJI.1830240331
148. M B, A S, B M, J H-H, T K, Mg R. Rapamycin promotes expansion of functional CD4+CD25+FOXP3+ regulatory T cells of both healthy subjects and type 1 diabetic patients. *Journal of immunology (Baltimore, Md : 1950)*. 2006/12// 2006;177(12):8338-8347. doi:10.4049/JIMMUNOL.177.12.8338
149. Allison AC, Eugui EM. Mycophenolate mofetil and its mechanisms of action. *Immunopharmacology*. 2000;47(2-3):85-118. doi:10.1016/S0162-3109(00)00188-0
150. Takaki T, Shimoda M. Pancreatic islet transplantation: toward definitive treatment for diabetes mellitus. *Global Health & Medicine*. 2020/8// 2020;2(4):200-200. doi:10.35772/GHM.2020.01057
151. Ryan EA, Paty BW, Senior PA, Shapiro AMJ, Address P. Risks and Side Effects of Islet Transplantation. *Current Diabetes Reports*. 2004;4:304-309.
152. Barlow AD, Nicholson ML, Herbert TP. Evidence for Rapamycin Toxicity in Pancreatic β-Cells and a Review of the Underlying Molecular Mechanisms. *Diabetes*. 2013/8// 2013;62(8):2674-2682. doi:10.2337/DB13-0106
153. Nir T, Melton DA, Dor Y. Recovery from diabetes in mice by beta cell regeneration. *The Journal of clinical investigation*. 2007/9// 2007;117(9):2553-2561. doi:10.1172/JCI32959
154. Desai TA, Tang Q. Islet encapsulation therapy - racing towards the finish line? *Nat Rev Endocrinol*. Nov 2018;14(11):630-632. doi:10.1038/s41574-018-0100-7
155. Eich T, Eriksson O, Lundgren T. Visualization of Early Engraftment in Clinical Islet Transplantation by Positron-Emission Tomography. *New England Journal of Medicine*. 2007/6// 2007;356(26):2754-2755. doi:10.1056/nejmc070201
156. J Coughlan A, R. McEachron K, E. Skube M, Muratore S, D. Bellin M, J Beilman G. Where's Waldo? Extrahepatic Site Options for Islet Transplantation. *OBM Transplantation 2019, Vol 3, Page 1*. 2019/10// 2019;3(1):1-1. doi:10.21926/OBM.TRANSPLANT.1901042

157. Salazar-Bañuelos A, Wright JR, Sigalet D, Benítez-Bribiesca L. Pancreatic islet transplantation into the bone marrow of the rat. *The American Journal of Surgery*. 2008/5// 2008;195(5):674-678. doi:10.1016/J.AMJSURG.2007.12.040
158. Cantarelli E, Citro A, Pellegrini S, et al. Transplant site influences the immune response after islet transplantation: Bone marrow versus liver. *Transplantation*. 2017;101(5):1046-1055. doi:10.1097/TP.0000000000001462
159. Maffi P, Nano R, Monti P, et al. Islet allotransplantation in the bone marrow of patients with type 1 diabetes: A pilot randomized trial. *Transplantation*. 2019/4// 2019;103(4):839-851. doi:10.1097/TP.0000000000002416
160. Nishimura R, Goto M, Sekiguchi S, Fujimori K, Ushiyama A, Satomi S. Assessment for revascularization of transplanted pancreatic islets at subcutaneous site in mice with a highly sensitive imaging system. Transplant Proc; 2011:3239-3240.
161. Saito T, Ohashi K, Utoh R, et al. Reversal of diabetes by the creation of neo-islet tissues into a subcutaneous site using islet cell sheets. *Transplantation*. 2011/12// 2011;92(11):1231-1236. doi:10.1097/TP.0b013e3182375835
162. Sakata N, Aoki T, Yoshimatsu G, et al. Strategy for clinical setting in intramuscular and subcutaneous islet transplantation. Diabetes/Metabolism Research and Reviews: Diabetes Metab Res Rev; 2014. p. 1-10.
163. Barkai U, Weir GC, Colton CK, et al. Enhanced oxygen supply improves islet viability in a new bioartificial pancreas. *Cell Transplantation*. 2013/8// 2013;22(8):1463-1476. doi:10.3727/096368912X657341
164. Carlsson PO, Espes D, Sedigh A, et al. Transplantation of macroencapsulated human islets within the bioartificial pancreas βAir to patients with type 1 diabetes mellitus. *American Journal of Transplantation*. 2018/7// 2018;18(7):1735-1744. doi:10.1111/AJT.14642
165. Anderson JM, Rodriguez A, Chang DT. Foreign body reaction to biomaterials. *Seminars in Immunology*. 2008/4// 2008;20(2):86-100. doi:10.1016/J.SMIM.2007.11.004
166. Kasoju N, Pátíková A, Wawrzynska E, et al. Bioengineering a pre-vascularized pouch for subsequent islet transplantation using VEGF-loaded polylactide capsules. *Biomaterials Science*. 2020/1// 2020;8(2):631-647. doi:10.1039/C9BM01280J
167. Vlahos AE, Talior-Volodarsky I, Kinney SM, Sefton MV. A scalable device-less biomaterial approach for subcutaneous islet transplantation. *Biomaterials*. 2021/2// 2021;269:120499-120499. doi:10.1016/J.BIOMATERIALS.2020.120499
168. Pepper AR, Gala-Lopez B, Pawlick R, Merani S, Kin T, Shapiro AMJ. A prevascularized subcutaneous device-less site for islet and cellular transplantation. *Nature Biotechnology*. 2015/05/01 2015;33(5):518-523. doi:10.1038/nbt.3211
169. Pepper AR, Gala-Lopez B, Pawlick R, Merani S, Kin T, Shapiro AMJ. A prevascularized subcutaneous device-less site for islet and cellular transplantation. *Nature Biotechnology 2015 33:5*. 2015/4// 2015;33(5):518-523. doi:10.1038/nbt.3211
170. Nalbach L, Roma LP, Schmitt BM, et al. Improvement of islet transplantation by the fusion of islet cells with functional blood vessels. *EMBO Molecular Medicine*. 2021/1// 2021;13(1)doi:10.15252/EMMM.202012616
171. Kinney SM, Ortaleza K, Vlahos AE, Sefton MV. Degradable methacrylic acid-based synthetic hydrogel for subcutaneous islet transplantation. *Biomaterials*. 2022/02/01/ 2022;281:121342. doi:https://doi.org/10.1016/j.biomaterials.2021.121342

172. Kuppan P, Kelly S, Seeberger K, et al. Bioabsorption of Subcutaneous Nanofibrous Scaffolds Influences the Engraftment and Function of Neonatal Porcine Islets. *Polymers*. 2022;14(6):1120. doi:10.3390/polym14061120
173. Foster GA, García AJ. Bio-synthetic materials for immunomodulation of islet transplants. *Advanced drug delivery reviews*. 2017/5// 2017;114:266-266. doi:10.1016/J.ADDR.2017.05.012
174. Dimitrioglou N, Kanelli M, Papageorgiou E, Karatzas T, Hatziavramidis D. Paving the way for successful islet encapsulation. *Drug Discovery Today*. 2019/3// 2019;24(3):737-748. doi:10.1016/J.DRUDIS.2019.01.020
175. Wu S, Wang L, Fang Y, Huang H, You X, Wu J. Advances in Encapsulation and Delivery Strategies for Islet Transplantation. *Advanced Healthcare Materials*. 2021/10// 2021;10(20):2100965-2100965. doi:10.1002/ADHM.202100965
176. Ludwig B, Ludwig S, Steffen A, et al. Favorable outcome of experimental islet xenotransplantation without immunosuppression in a nonhuman primate model of diabetes. *Proceedings of the National Academy of Sciences*. 2017/10// 2017;114(44):11745-11750. doi:10.1073/PNAS.1708420114
177. Song S, Blaha C, Moses W, et al. An intravascular bioartificial pancreas device (iBAP) with silicon nanopore membranes (SNM) for islet encapsulation under convective mass transport. *Lab on a chip*. 2017/5// 2017;17(10):1778-1778. doi:10.1039/C7LC00096K
178. Choe G, Park J, Park H, Lee JY. Hydrogel Biomaterials for Stem Cell Microencapsulation. *Polymers 2018, Vol 10, Page 997*. 2018/9// 2018;10(9):997-997. doi:10.3390/POLYM10090997
179. Zhang J, Zhu Y, Song J, et al. Rapid and Long-Term Glycemic Regulation with a Balanced Charged Immune-Evasive Hydrogel in T1DM Mice. *Advanced Functional Materials*. 2019/5// 2019;29(19):1900140-1900140. doi:10.1002/ADFM.201900140
180. Holdcraft RW, Gazda LS, Circle L, et al. Enhancement of in vitro and in vivo function of agarose-encapsulated porcine islets by changes in the islet microenvironment. *Cell transplantation*. 2014;23(8):929-944. doi:10.3727/096368913X667033
181. McEwan K, Padavan DT, Ellis C, et al. Collagen-chitosan-laminin hydrogels for the delivery of insulin-producing tissue. *Journal of tissue engineering and regenerative medicine*. 2016/10// 2016;10(10):E397-E408. doi:10.1002/TERM.1829
182. Qi Z, Yamamoto C, Imori N, et al. Immunoisolation effect of polyvinyl alcohol (PVA) macroencapsulated islets in type 1 diabetes therapy. *Cell Transplantation*. 2012/3// 2012;21(2-3):525-534. doi:10.3727/096368911X605448
183. Dufour JM, Rajotte RV, Zimmerman M, et al. Development of an ectopic site for islet transplantation, using biodegradable scaffolds. *Tissue engineering*. 2005/9// 2005;11(9-10):1323-1331. doi:10.1089/TEN.2005.11.1323
184. Omami M, McGarrigle JJ, Reedy M, et al. Islet Microencapsulation: Strategies and Clinical Status in Diabetes. *Current Diabetes Reports*. 2017/7// 2017;17(7):1-7. doi:10.1007/S11892-017-0877-0/TABLES/1
185. Krishnan R, Alexander M, Robles L, Foster CE, Lakey JRT. Islet and stem cell encapsulation for clinical transplantation. *Review of Diabetic Studies*. 2014;11(1):84-101. doi:10.1900/RDS.2014.11.84
186. Olabisi RM. Cell microencapsulation with synthetic polymers. *Journal of Biomedical Materials Research Part A*. 2015/2// 2015;103(2):846-859. doi:10.1002/JBM.A.35205
187. Stabler CL, Giraldo JA, Berman DM, et al. Transplantation of PEGylated islets enhances therapeutic efficacy in a diabetic nonhuman primate model. *American journal of transplantation*

: official journal of the American Society of Transplantation and the American Society of Transplant Surgeons. 2020/3// 2020;20(3):689-700. doi:10.1111/AJT.15643
188. Zhi ZL, Kerby A, King AJF, Jones PM, Pickup JC. Nano-scale encapsulation enhances allograft survival and function of islets transplanted in a mouse model of diabetes. *Diabetologia*. 2012/4// 2012;55(4):1081-1090. doi:10.1007/S00125-011-2431-Y
189. Barra JM, Kozlovskaya V, Kharlampieva E, Tse HM. Localized Immunosuppression With Tannic Acid Encapsulation Delays Islet Allograft and Autoimmune-Mediated Rejection. *Diabetes*. 2020/9// 2020;69(9):1948-1960. doi:10.2337/DB20-0248
190. Paez-Mayorga J, Campa-Carranza JN, Capuani S, et al. Implantable niche with local immunosuppression for islet allotransplantation achieves type 1 diabetes reversal in rats. *Nature Communications*. 2022/12/26 2022;13(1):7951. doi:10.1038/s41467-022-35629-z
191. Wang X, Brown NK, Wang B, et al. Local Immunomodulatory Strategies to Prevent Allo-Rejection in Transplantation of Insulin-Producing Cells. *Advanced Science*. 2021/9// 2021;8(17):2003708-2003708. doi:10.1002/ADVS.202003708
192. Long G, Zhang G, Zhang F, et al. Cotransplantation of Mesenchymal Stem Cells and Immature Dendritic Cells Potentiates the Blood Glucose Control of Islet Allografts. *BioMed Research International*. 2017;2017doi:10.1155/2017/4107943
193. Krzystyniak A, Gołąb K, Witkowski P, Trzonkowski P. Islet cell transplant and the incorporation of Tregs. *Current opinion in organ transplantation*. 2014;19(6):610-610. doi:10.1097/MOT.0000000000000130
194. Mital P, Kaur G, Dufour JM. Immunoprotective Sertoli cells: making allogeneic and xenogeneic transplantation feasible. *Reproduction*. 2010/3// 2010;139(3):495-504. doi:10.1530/REP-09-0384
195. Hotaling NA, Tang L, Irvine DJ, Babensee JE. Biomaterial Strategies for Immunomodulation. *http://dxdoiorg/101146/annurev-bioeng-071813-104814*. 2015/12// 2015;17:317-349. doi:10.1146/ANNUREV-BIOENG-071813-104814
196. Hubbell JA, Thomas SN, Swartz MA. Materials engineering for immunomodulation. *Nature 2009 462:7272*. 2009/11// 2009;462(7272):449-460. doi:10.1038/nature08604
197. Reddy MSB, Ponnamma D, Choudhary R, Sadasivuni KK. A Comparative Review of Natural and Synthetic Biopolymer Composite Scaffolds. *Polymers 2021, Vol 13, Page 1105*. 2021/3// 2021;13(7):1105-1105. doi:10.3390/POLYM13071105
198. Makadia HK, Siegel SJ. Poly Lactic-co-Glycolic Acid (PLGA) as Biodegradable Controlled Drug Delivery Carrier. *Polymers 2011, Vol 3, Pages 1377-1397*. 2011/8// 2011;3(3):1377-1397. doi:10.3390/POLYM3031377
199. Liu JMH, Zhang J, Zhang X, et al. Transforming growth factor-beta 1 delivery from microporous scaffolds decreases inflammation post-implant and enhances function of transplanted islets. *Biomaterials*. 2016/2// 2016;80:11-19. doi:10.1016/J.BIOMATERIALS.2015.11.065
200. Awojoodu AO, Ogle ME, Sefcik LS, et al. Sphingosine 1-phosphate receptor 3 regulates recruitment of anti-inflammatory monocytes to microvessels during implant arteriogenesis. *Proceedings of the National Academy of Sciences of the United States of America*. 2013;110(34):13785-13790. doi:10.1073/PNAS.1221309110
201. Frei AW, Li Y, Jiang K, Buchwald P, Stabler CL. Local delivery of fingolimod from three-dimensional scaffolds impacts islet graft efficacy and microenvironment in a murine diabetic model. *Journal of Tissue Engineering and Regenerative Medicine*. 2018/2// 2018;12(2):393-404. doi:10.1002/TERM.2464

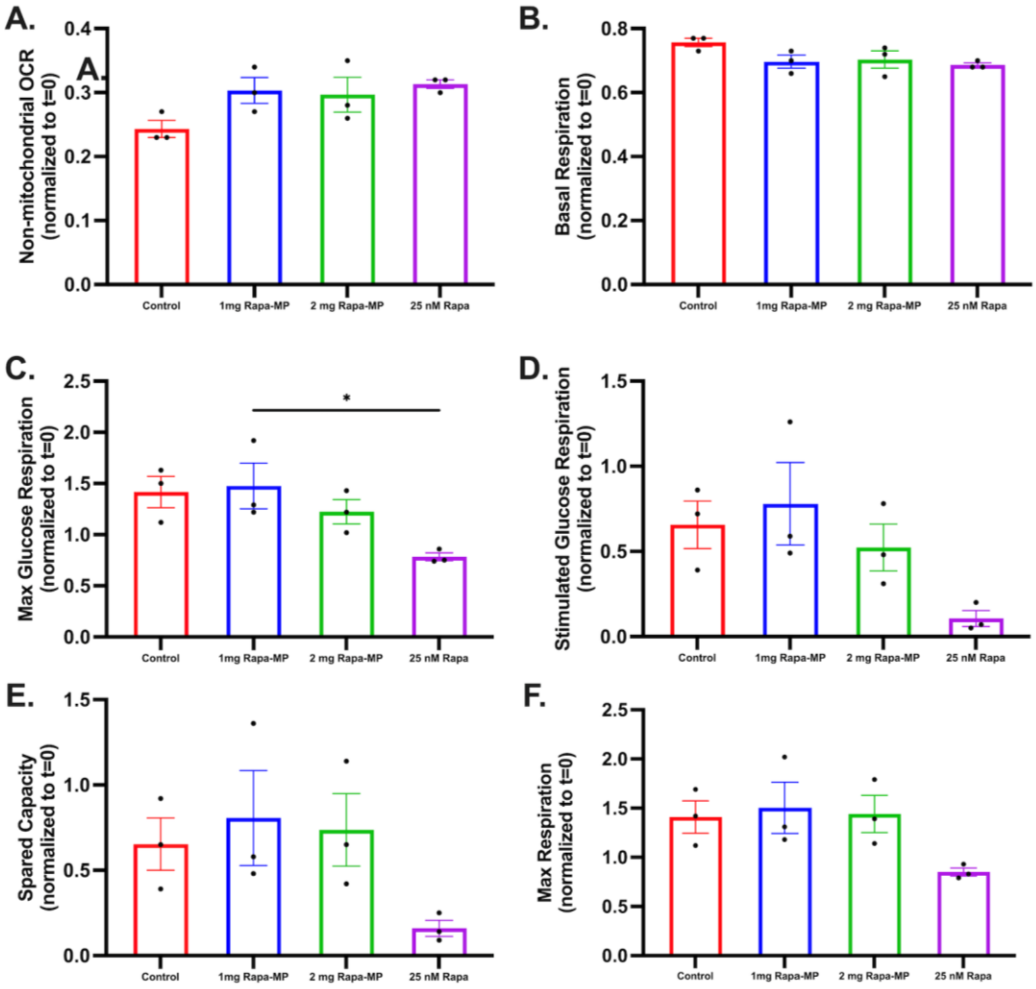
202. Bale S, Khurana A, Reddy ASS, Singh M, Godugu C. Overview on Therapeutic Applications of Microparticulate Drug Delivery Systems. *Critical Reviews™ in Therapeutic Drug Carrier Systems*. 2016;33(4):309-361. doi:10.1615/CRITREVTHERDRUGCARRIERSYST.2016015798
203. De Jong WH, Borm PJA. Drug delivery and nanoparticles: Applications and hazards. *International Journal of Nanomedicine*. 2008/6// 2008;3(2):133-149. doi:10.2147/IJN.S596
204. Kuppan P, Kelly S, Polishevska K, et al. Co-localized immune protection using dexamethasone-eluting micelles in a murine islet allograft model. *American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons*. 2020/3// 2020;20(3):714-725. doi:10.1111/AJT.15662
205. Pathak S, Regmi S, Gupta B, et al. Single synchronous delivery of fk506-loaded polymeric microspheres with pancreatic islets for the successful treatment of streptozocin-induced diabetes in mice. *Drug Delivery*. 2017;24(1):1350-1359. doi:10.1080/10717544.2017.1377317/SUPPL_FILE/IDRD_A_1377317_SM1374.DOCX
206. Fan Y, Zheng X, Ali Y, Berggren PO, Loo SCJ. Local release of rapamycin by microparticles delays islet rejection within the anterior chamber of the eye. *Scientific Reports 2019 9:1*. 2019/3// 2019;9(1):1-9. doi:10.1038/s41598-019-40404-0
207. Bryant J, Hlavaty KA, Zhang X, et al. Nanoparticle delivery of donor antigens for transplant tolerance in allogeneic islet transplantation. *Biomaterials*. 2014/10// 2014;35(31):8887-8894. doi:10.1016/J.BIOMATERIALS.2014.06.044
208. MacDonald AS. A worldwide, phase III, randomized, controlled, safety and efficacy study of a sirolimus/cyclosporine regimen for prevention of acute rejection in recipients of primary mismatched renal allografts. *Transplantation*. Jan 27 2001;71(2):271-80. doi:10.1097/00007890-200101270-00019
209. Kahan BD. Efficacy of sirolimus compared with azathioprine for reduction of acute renal allograft rejection: a randomised multicentre study. The Rapamune US Study Group. *Lancet*. Jul 15 2000;356(9225):194-202. doi:10.1016/s0140-6736(00)02480-6
210. Johnston O, Rose CL, Webster AC, Gill JS. Sirolimus is associated with new-onset diabetes in kidney transplant recipients. *J Am Soc Nephrol*. Jul 2008;19(7):1411-8. doi:10.1681/asn.2007111202
211. Holdaas H, Rostaing L, Serón D, et al. Conversion of long-term kidney transplant recipients from calcineurin inhibitor therapy to everolimus: a randomized, multicenter, 24-month study. *Transplantation*. Aug 27 2011;92(4):410-8. doi:10.1097/TP.0b013e318224c12d
212. Schena FP, Pascoe MD, Alberu J, et al. Conversion from calcineurin inhibitors to sirolimus maintenance therapy in renal allograft recipients: 24-month efficacy and safety results from the CONVERT trial. *Transplantation*. Jan 27 2009;87(2):233-42. doi:10.1097/TP.0b013e3181927a41
213. Asleh R, Briasoulis A, Kremers WK, et al. Long-Term Sirolimus for Primary Immunosuppression in Heart Transplant Recipients. *J Am Coll Cardiol*. Feb 13 2018;71(6):636-650. doi:10.1016/j.jacc.2017.12.005
214. Manavitehrani I, Fathi A, Badr H, Daly S, Negahi Shirazi A, Dehghani F. Biomedical Applications of Biodegradable Polyesters. *Polymers*. 2016;8(1):20.
215. Kuppan P, Wong J, Kelly S, et al. Long-Term Survival and Induction of Operational Tolerance to Murine Islet Allografts by Co-Transplanting Cyclosporine A Microparticles and CTLA4-Ig. *Pharmaceutics*. Aug 25 2023;15(9)doi:10.3390/pharmaceutics15092201

216. Kuppan P, Kelly S, Polishevska K, et al. Co-localized immune protection using dexamethasone-eluting micelles in a murine islet allograft model. *American Journal of Transplantation*. 2020/03/01/ 2020;20(3):714-725. doi:https://doi.org/10.1111/ajt.15662
217. Ankrum JA, Miranda OR, Ng KS, Sarkar D, Xu C, Karp JM. Engineering cells with intracellular agent–loaded microparticles to control cell phenotype. *Nature Protocols*. 2014/02/01 2014;9(2):233-245. doi:10.1038/nprot.2014.002
218. Woodward KB, Zhao H, Shrestha P, et al. Pancreatic islets engineered with a FasL protein induce systemic tolerance at the induction phase that evolves into long-term graft-localized immune privilege. *Am J Transplant*. May 2020;20(5):1285-1295. doi:10.1111/ajt.15747
219. Lee S-J, Kim H-J, Byun N-r, Park C-G. Donor-Specific Regulatory T Cell-Mediated Immune Tolerance in an Intrahepatic Murine Allogeneic Islet Transplantation Model with Short-Term Anti-CD154 mAb Single Treatment. *Cell Transplantation*. 2020;29:0963689720913876. doi:10.1177/0963689720913876
220. Zammit NW, Walters SN, Seeberger KL, O'Connell PJ, Korbutt GS, Grey ST. A20 as an immune tolerance factor can determine islet transplant outcomes. *JCI Insight*. Nov 1 2019;4(21)doi:10.1172/jci.insight.131028
221. Coenen JJ, Koenen HJ, van Rijssen E, Hilbrands LB, Joosten I. Rapamycin, and not cyclosporin A, preserves the highly suppressive CD27+ subset of human CD4+CD25+ regulatory T cells. *Blood*. Feb 1 2006;107(3):1018-23. doi:10.1182/blood-2005-07-3032
222. Veilleux A, Houde VP, Bellmann K, Marette A. Chronic Inhibition of the mTORC1/S6K1 Pathway Increases Insulin-Induced PI3K Activity but Inhibits Akt2 and Glucose Transport Stimulation in 3T3-L1 Adipocytes. *Molecular Endocrinology*. 2010;24(4):766-778. doi:10.1210/me.2009-0328
223. Vu MD, Qi S, Xu D, et al. Synergistic effects of mycophenolate mofetil and sirolimus in prevention of acute heart, pancreas, and kidney allograft rejection and in reversal of ongoing heart allograft rejection in the rat. *Transplantation*. Dec 27 1998;66(12):1575-80. doi:10.1097/00007890-199812270-00002
224. Brook NR, Waller JR, Bicknell GR, Nicholson ML. Cyclosporine and rapamycin act in a synergistic and dose-dependent manner in a model of immunosuppressant-induced kidney damage. *Transplant Proc*. Mar 2005;37(2):837-8. doi:10.1016/j.transproceed.2004.12.147
225. Vu MD, Qi S, Xu D, et al. Tacrolimus (FK506) and sirolimus (rapamycin) in combination are not antagonistic but produce extended graft survival in cardiac transplantation in the rat. *Transplantation*. Dec 27 1997;64(12):1853-6. doi:10.1097/00007890-199712270-00039
226. Chen L, Flies DB. Molecular mechanisms of T cell co-stimulation and co-inhibition. *Nature Reviews Immunology*. 2013/04/01 2013;13(4):227-242. doi:10.1038/nri3405
227. Jenkins MK, Schwartz RH. Antigen presentation by chemically modified splenocytes induces antigen-specific T cell unresponsiveness in vitro and in vivo. *J Exp Med*. Feb 1 1987;165(2):302-19. doi:10.1084/jem.165.2.302
228. Bluestone JA, St. Clair EW, Turka LA. CTLA4Ig: Bridging the Basic Immunology with Clinical Application. *Immunity*. 2006/03/01/ 2006;24(3):233-238. doi:https://doi.org/10.1016/j.immuni.2006.03.001
229. Lee SJ, Kim HJ, Byun NR, Park CG. Donor-Specific Regulatory T Cell-Mediated Immune Tolerance in an Intrahepatic Murine Allogeneic Islet Transplantation Model with Short-Term Anti-CD154 mAb Single Treatment. *Cell Transplant*. Jan-Dec 2020;29:963689720913876. doi:10.1177/0963689720913876

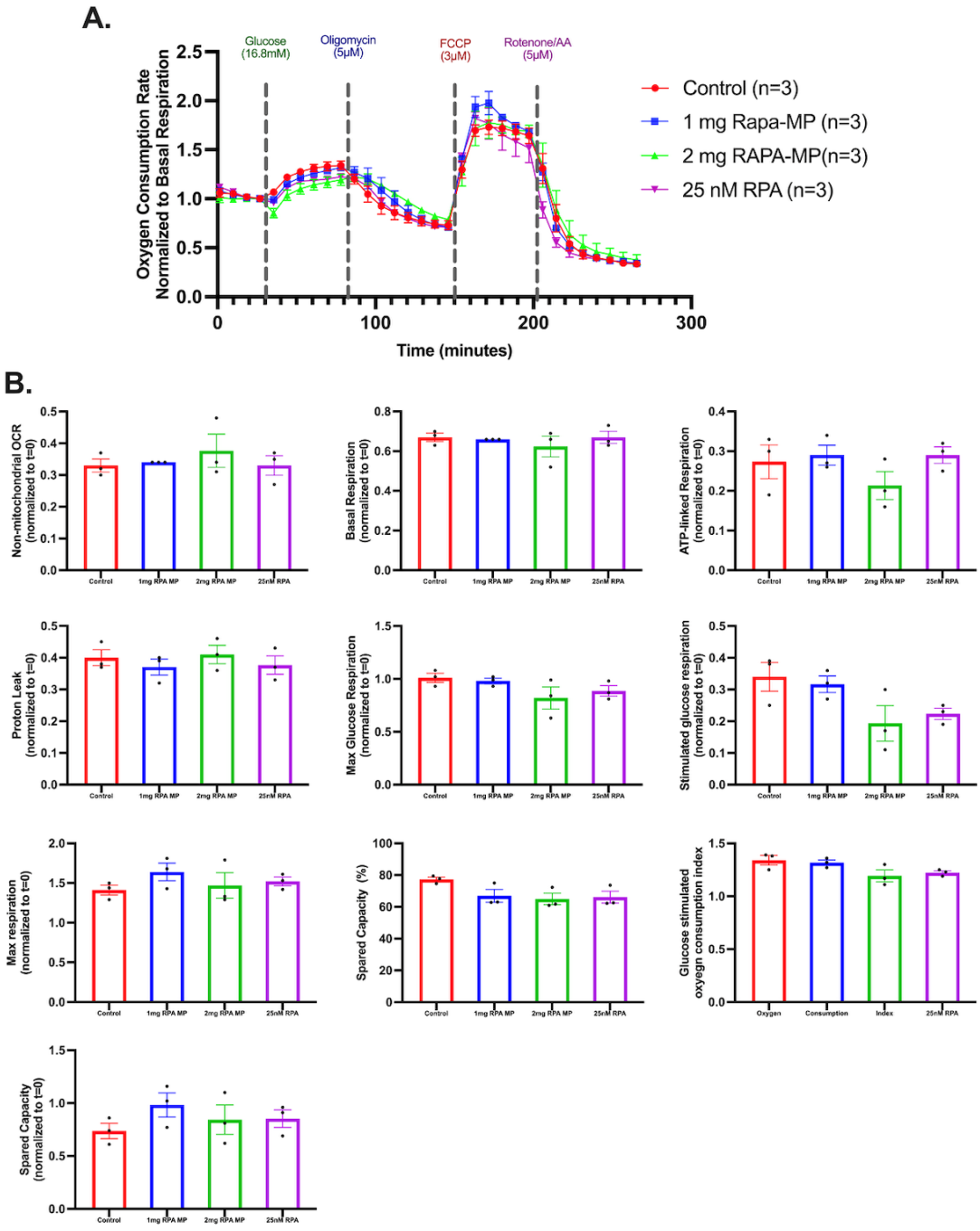
230. Ma Y, Lin BR, Lin B, et al. Pharmacokinetics of CTLA4Ig fusion protein in healthy volunteers and patients with rheumatoid arthritis. *Acta Pharmacol Sin*. Mar 2009;30(3):364-71. doi:10.1038/aps.2009.13
231. Sachs DH. Transplant tolerance: bench to bedside--26th annual Samuel Jason Mixter Lecture. *Arch Surg*. May 2011;146(5):501-5. doi:10.1001/archsurg.2011.101
232. Kenley RA, Lee MO, Mahoney TR, Sanders LM. Poly (lactide-co-glycolide) decomposition kinetics in vivo and in vitro. *Macromolecules*. 1987;20(10):2398-2403.
233. Fan Y, Zheng X, Ali Y, Berggren P-O, Loo SCJ. Local release of rapamycin by microparticles delays islet rejection within the anterior chamber of the eye. *Scientific Reports*. 2019/03/08 2019;9(1):3918. doi:10.1038/s41598-019-40404-0
234. Shapiro AM, Ricordi C, Hering BJ, et al. International trial of the Edmonton protocol for islet transplantation. *N Engl J Med*. Sep 28 2006;355(13):1318-30. doi:10.1056/NEJMoa061267
235. Alwahsh SM, Qutachi O, Starkey Lewis PJ, et al. Fibroblast growth factor 7 releasing particles enhance islet engraftment and improve metabolic control following islet transplantation in mice with diabetes. *American Journal of Transplantation*. 2021/9// 2021;21(9):2950-2963. doi:10.1111/AJT.16488
236. Shapiro AMJ, Lakey JRT, Ryan EA, et al. Islet Transplantation in Seven Patients with Type 1 Diabetes Mellitus Using a Glucocorticoid-Free Immunosuppressive Regimen. *http://dxdoiorg/101056/NEJM200007273430401*. 2000/7// 2000;343(4):230-238. doi:10.1056/NEJM200007273430401
237. Santos AS, Cunha-Neto E, Gonfinetti NV, et al. Prevalence of Inflammatory Pathways Over Immuno-Tolerance in Peripheral Blood Mononuclear Cells of Recent-Onset Type 1 Diabetes. *Front Immunol*. 2021;12:765264. doi:10.3389/fimmu.2021.765264
238. Shapiro AMJ, Lakey JRT, Ryan EA, et al. Islet Transplantation in Seven Patients with Type 1 Diabetes Mellitus Using a Glucocorticoid-Free Immunosuppressive Regimen. *http://dxdoiorg/101056/NEJM200007273430401*. 2009/8// 2009;343(4):230-238. doi:10.1056/NEJM200007273430401

APPENDIX

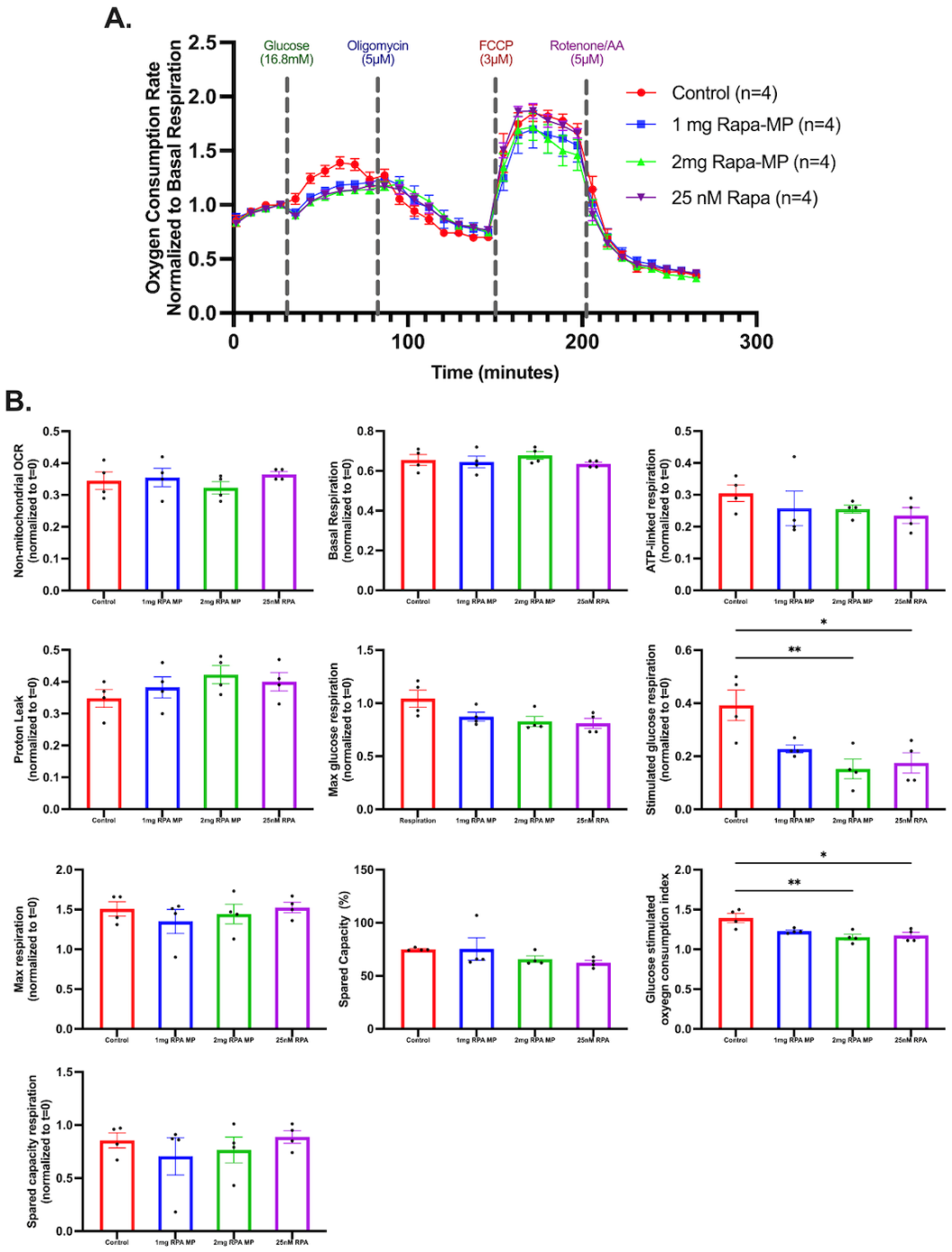
Appendix A-1: Supplemental Figures and Tables



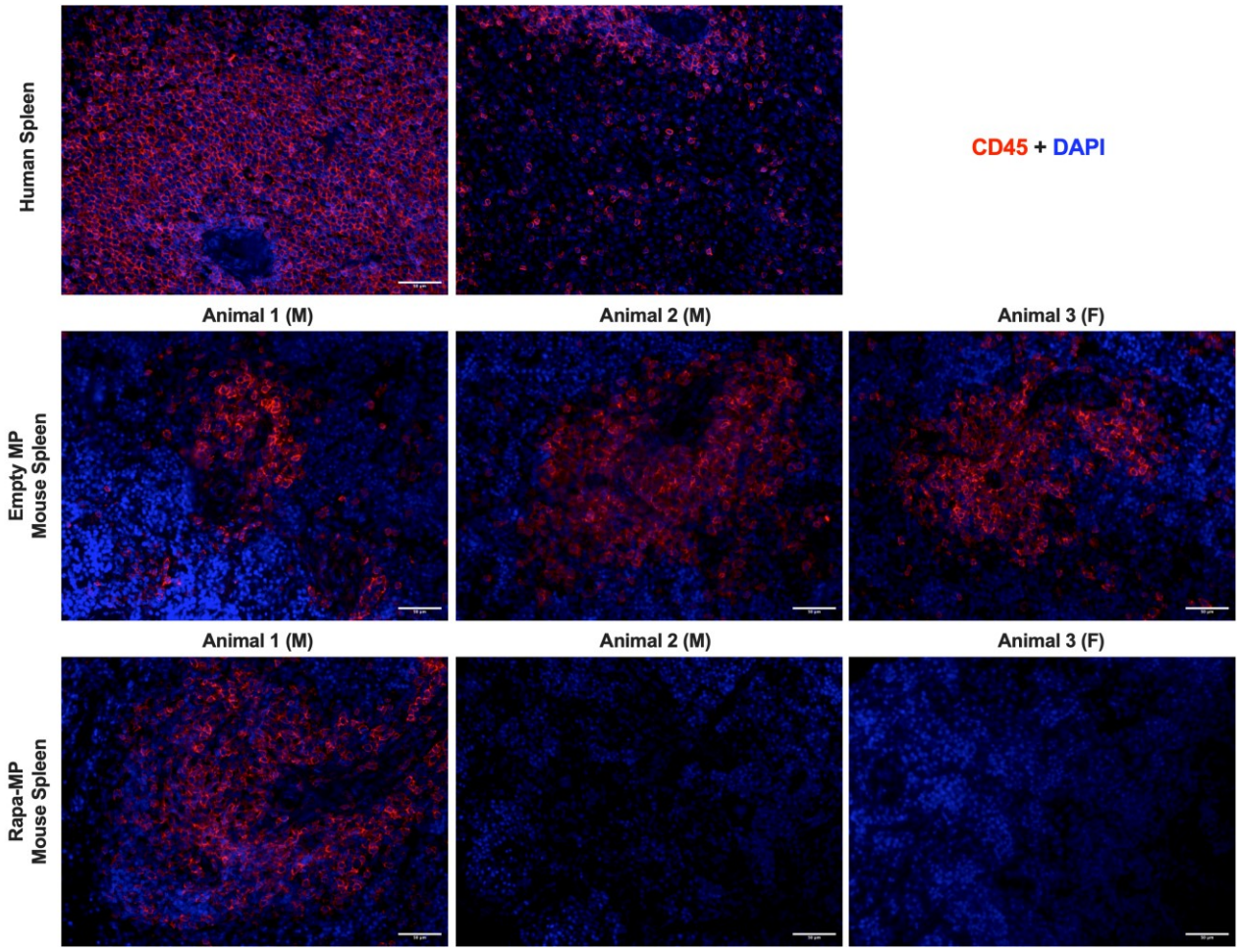
Supplementary Figure 1: Generated parameters from changes in the oxygen consumption rate of a single donor human islets examined under the Extracellular Flux Analyzer CF24. From human donor islets generated from **Figure 2.2.** Data is normalized to basal respiration. *p<0.05



Supplementary Figure 2: Respiration and mitochondrial function of a single donor human islets co-cultured for 24 hours with rapa-MP or rapa. All data is represented as mean +/- SEM and normalized to basal respiration. **A)** Oxygen consumption rate of islets following sequential stimulation with glucose and electron transport chain modulators. Dotted grey lines indicate instance of agent injected into the port. **B)** Generated parameters from changes in the oxygen consumption rate.



Supplementary Figure 3: Respiration and mitochondrial function of a single donor human islets co-cultured for 24 hours with rapa-MP or rapa. All data is represented as mean +/- SEM and normalized to basal respiration. **A)** Oxygen consumption rate of islets following sequential stimulation with glucose and electron transport chain modulators. Dotted grey lines indicate instance of agent injected into the port. **B)** Generated parameters from changes in the oxygen consumption rate. *p<0.05 and **p<0.01



Supplementary Figure 4: Immunochemistry of NSG-MHC I/II DKO mouse spleens collected at 6 w post-injection with human PBMCs, with human spleens as reference. Mice were transplanted with empty MP or 0.1 mg/kg rapa-MP + human islets at the time of PBMC injection. CD45+ cells (red), nuclear DNA (blue)

Supplementary Table 1: Donor characteristics of human islets and PBMC.

Experiment	Donor Age	Sex	BMI	Purity (%)	Type
Seahorse XF (Figure 2.2)	**53**	**F**	**12.8**	**95**	**Islets**
Seahorse XF (Supplementary Figure 2)	**61**	**F**	**36.1**	**95**	**Islets**
Seahorse XF (Supplementary Figure 3)	**64**	**M**	**40.2**	**80**	**Islets**
NSG Transplants (Figure 2.13, 2.14)	**53**	**M**	**34.5**	**95**	**Islets**
NSG Transplants (Figure 2.13, 2.14 and Supplementary Figure 4)	**54**	**M**	**28.1**	**50**	**Islets**
NSG Transplants (Figure 2.13, 2.14)	**32**	**F**	**26.4**	**98 % viability**	**PBMCs**
NSG Transplants (Figure 2.13,2.14 and Supplementary Figure 4)	**43**	**F`**	**29.0**	**99.7% viability**	**PBMCs**

Supplementary Table 2: Antibodies used flow cytometry.

Antibody	Cat. Number	Working dilution	Isotype
Anti-mouse CD45	BioLegend, 103112	1/ 5000	Biolegend, 400612
Anti-human CD45	Biolegend, 368506	1/ 200	BioLegend, 400148
Anti-human CD3	Biolegend, 344872	1/ 100	BioLegend, 400164
Anti-human CD4	BioLegend, 300538	1/ 20	Biolegend, 400110
Anti-human CD8	BioLegend, 344748	1/ 20	Biolegend, 400158
Anti FoxP3	BioLegend 320008	1/ 20	BioLegend, 400140

Appendix A-2

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Long-Term Survival and Induction of Operational Tolerance to Murine Islet Allografts by Co-Transplanting Cyclosporine A Microparticles and CTLA4-Ig

by Purushothaman Kuppan [1,2], Jordan Wong [1,2], Sandra Kelly [1,2], Jiaxin Lin [1,2], Jessica Worton [1,2], Chelsea Castro [1,2], Joy Paramor [1,2], Karen Seeberger [1,2], Nerea Cuesta-Gomez [1,2], Colin C. Anderson [1,2], Gregory S. Korbutt [1,2,*] and Andrew R. Pepper [1,2,*]

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(This article belongs to the Topic **Recent Advancement in Biotechnology and Drug Development Using Cutting-Edge Platforms**)

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Abstract

One strategy to prevent islet rejection is to create a favorable immune-protective local environment at the transplant site. Herein, we utilize localized cyclosporine A (CsA) delivery to islet grafts via poly(lactic-co-glycolic acid) (PLGA) microparticles to attenuate allograft rejection. CsA-eluting PLGA microparticles were prepared using a single emulsion (oil-in-water) solvent evaporation technique. CsA microparticles alone significantly delayed islet allograft rejection compared to islets alone ($p < 0.05$). Over 50% (6/11) of recipients receiving CsA microparticles and short-term cytotoxic T lymphocyte-associated antigen 4-Ig (CTLA4-Ig) therapy displayed prolonged allograft survival for 214 days, compared to 25% (2/8) receiving CTLA4-Ig alone. CsA microparticles alone and CsA microparticles + CTLA4-Ig islet allografts exhibited reduced T-cell ($CD4^+$ and $CD8^+$ cells, $p < 0.001$) and macrophage ($CD68^+$ cells, $p < 0.001$) infiltration compared to islets alone. We observed the reduced mRNA expression of proinflammatory cytokines (*IL-6*, *IL-10*, *INF-γ*, and *TNF-α*; $p < 0.05$) and chemokines (*CCL2*, *CCL5*, *CCL22*, and *CXCL10*; $p < 0.05$) in CsA microparticles + CTLA4-Ig allografts compared to islets alone. Long-term islet allografts contained $insulin^+$ and intra-graft $FoxP3^+$ T regulatory cells. The rapid rejection of third-party skin grafts (C3H) in islet allograft recipients suggests that CsA microparticles + CTLA4-Ig therapy induced operational tolerance. This study demonstrates that localized CsA drug delivery plus short-course systemic immunosuppression promotes an immune protective transplant niche for allogeneic islets.

Keywords: **type 1 diabetes**; **localized drug delivery**; **cyclosporine A**; **murine islet allograft**; **islet transplantation**; **CTLA4-Ig**

Appendix A-2: Abstract of the publication in *Pharmaceutics,* "**Long-Term Survival and Induction of Operational Tolerance to Murine Islet Allografts by Co-Transplanting Cyclosporine A Microparticles and CTLA4-Ig**" by P. Kuppan, J. Wong, S. Kelly, J. Lin, J. Worton, C. Castro, J Parmor, K. Seeberger, N. Cuesta-Gomez, C. Anderson, G. Korbutt, and A. Pepper.

Appendix A-3

New Results

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HumanIslets: An integrated platform for human islet data access and analysis

Jessica D. Ewald, Yao Lu, Cara E. Ellis, Jessica Worton, Jelena Kolic, Shugo Sasaki, Dahai Zhang, Theodore dos Santos, Aliya F. Spigelman, Austin Bautista, Xiao-Qing Dai, James G. Lyon, Nancy P. Smith, Jordan M. Wong, Varsha Rajesh, Han Sun, Seth A. Sharp, Jason C. Rogalski, Renata Moravcova, Haoning H Cen, Jocelyn E. Manning Fox, HI-DAS Consortium, Ella Atlas, Jennifer E. Bruin, Erin E. Mulvihill, C. Bruce Verchere, Leonard J. Foster, Anna L. Gloyn, James D. Johnson, Andrew R. Pepper, Francis C. Lynn, Jianguo Xia, Patrick E. MacDonald

doi: https://doi.org/10.1101/2024.06.19.599613

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SUMMARY

Comprehensive molecular and cellular phenotyping of human islets can enable deep mechanistic insights for diabetes research. We established the Human Islet Data Analysis and Sharing (HI-DAS) consortium to advance goals in accessibility, usability, and integration of data from human islets isolated from donors with and without diabetes at the Alberta Diabetes Institute (ADI) IsletCore. Here we introduce HumanIslets.com, an open resource for the research community. This platform, which presently includes data on 547 human islet donors, allows users to access linked datasets describing molecular profiles, islet function and donor phenotypes, and to perform various statistical and functional analyses at the donor, islet and single-cell levels. As an example of the analytic capacity of this resource we show a dissociation between cell culture effects on transcript and protein expression, and an approach to correct for exocrine contamination found in hand-picked islets. Finally, we provide an example workflow and visualization that highlights links between type 2 diabetes status, SERCA3b Ca^{2+}-ATPase levels at the transcript and protein level, insulin secretion and islet cell phenotypes. HumanIslets.com provides a growing and adaptable set of resources and tools to support the metabolism and diabetes research community.

Appendix A-3: Abstract of an article submitted as preprint in bioRxiv titled **"HumanIslets: An integrated platform for human islet data access and analysis"** by Ewald et. al.

Appendix A-4

Title page of manuscript submitted for peer review publication titled “Pre-transplant Aerobic Exercise Improves Glycemic Outcomes After Marginal Islet Mass Transplantation in Rats” by Wong et. al and is awaiting a decision.

Pre-transplant Aerobic Exercise Improves Glycemic Outcomes After Marginal Islet Mass Transplantation in Rats

Jordan M. Wong BSc[1], Seyed Amirhossein Tabatabaei Dakhili PhD[2], Purushothaman Kuppan PhD[1], Joy Paramor[1], John R. Ussher PhD[2], Caroline Richard PhD[3], C W James Melling PhD[4], Corbin Nitz BKin[5], **Jane E. Yardley PhD[6], Andrew R. Pepper PhD[1*]**

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