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Local Dexamethasone Administration Delays Allogeneic Islet Graft Rejection in the Anterior Chamber of the Eye of Non-Human Primates

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Abstract

Pancreatic islet transplantation into the anterior chamber of the eye (ACE) has been shown to improve glycemic control and metabolic parameters of diabetes in both murine and primate models. This novel transplantation site also allows the delivery of therapeutic agents, such as immunosuppressive drugs, locally to prevent islet graft rejection and circumvent unwanted systemic side effects. Local intravitreal administration of micronized dexamethasone implant was performed prior to allogeneic islet transplantation into the ACEs of non-human primates. Two study groups were observed namely allogeneic graft without immunosuppression (n = 4 eyes) and allogeneic graft with local immunosuppression (n = 8 eyes). Survival of islet grafts and dexamethasone concentration in the ACE were assessed in parallel for 24 weeks. Allogeneic islet grafts with local dexamethasone treatment showed significantly better survival than those with no immunosuppression (median survival time- 15 weeks vs 3 weeks, log-rank test $p<0.0001$). Around 73% of the grafts still survived at week 10 with a single local dexamethasone implant, where the control group showed no graft survival. Dexamethasone treated islet grafts revealed a good functional response to high glucose stimulation despite there was a transient suppression of insulin secretion from week 8 to 12. Our findings show a significant improvement of allografts survival in the ACE with local dexamethasone treatment. These results highlight the feasibility of local administration of pharmacological compounds in the ACE to improve islet graft survival and function. By eliminating the need for systemic immunosuppression, these findings may impact clinical islet transplantation in the treatment of diabetes, and the ACE may serve as a novel therapeutic islet transplantation site with high potential for local pharmacological intervention.

Keywords

non-human primates, islet transplantation, anterior chamber of the eye, intra-ocular transplant, dexamethasone, local immunosuppression

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Introduction

Pancreatic islet transplantation has been shown to improve glycemic control, insulin dependency, severe hypoglycaemia unawareness, and increase plasma C-peptide levels in type-1 diabetic (T1DM) patients, thereby improving quality of life[1–7]. It was also shown to improve type-2 diabetic (T2DM) conditions in our previous study in a high-fat diet induced type-2 diabetic non-human primate (NHP) model[8]. Currently, intraocular islet transplantation is being evaluated in Phase I clinical trial. Among different islet transplantation sites, the anterior chamber of the eye (ACE) offers a unique confined oxygen-rich and immune-privileged space which prevents transplanted islets from being immediately destroyed. However, the immune privilege is disrupted once vascularisation occurs, which allows the host immune cells to interact with the graft directly. We have previously shown that transplantation of pancreatic islets into the ACE is feasible and efficacious in T1DM and T2DM pre-clinical models using systemic immunosuppression[8–11].

Like other organ transplantations, immunosuppression is necessary simultaneously with pancreatic islet transplantation to alleviate the host's T-cells mediated responses and inflammatory reactions that ultimately contribute to graft rejection. Lifelong immunosuppression treatment is often needed to reduce tissue rejection and thereby maintaining the grafts[12]. However, undesirable side effects of systemic immunosuppressive drugs and stringent criteria to receive the islet transplant have been a hurdle in the islet transplantation field. Complications of long-term systemic immunosuppression include susceptibility to infection, renal dysfunction, and increased risk of cancer, not to mention that most of the immunosuppressive agents have negative effects on islet graft's function[13]. These adverse effects represent a significant barrier to the widespread use of pancreatic islet transplantation to manage insulin deficiency in diabetes. Hence, there is a call for strategies to avoid the necessity of systemic immunosuppression. One potential solution is the use of local immunosuppression at the site of transplantation to prevent graft rejection. Nonetheless, the success of local delivery of immunosuppressive drugs is critically dependent on the anatomy and accessibility of the transplantation site. In this context, the ACE offers high feasibility to use local therapeutic approaches to circumvent the immunogenic attacks upon the transplanted islets.

In addition, local delivery of anti-inflammatory and immunosuppressive drugs has been successfully used in ophthalmology to treat eye diseases, including uveitis, cornea graft rejection, and macular oedema. Dexamethasone, a potent glucocorticoid, is one of the commonly used compounds[14]. Furthermore, dexamethasone was shown to suppress inflammatory reactions and host immune responses to the allogeneic pancreatic islet grafts. It was reported that allogeneic islets transplanted together with dexamethasone scaffolds locally in the omentum and epididymal fat pad showed improved engraftment and survival of the islets in murine models[15–17]. In the present study, we used a sustained-release dexamethasone implant (Ozurdex, Allergan, Dublin, Ireland) for intravitreal injection, manufactured from a water-soluble biodegradable copolymer of lactic acid and glycolic acid. It was approved for the treatment of non-infectious uveitis, macular oedema arising from diabetic eye diseases and retinal vein occlusion, and the outcomes from the clinical trials showed an efficacy for up to 4 months[18].

From our previous studies, we reported that intraocular syngeneic and autologous islet grafts could be maintained for several months without deterioration in the absence of an immunosuppression regime[9,19,20]. On the other hand, allogeneic islet grafts showed a marked deterioration after a few weeks of transplantation, especially once vascularisation takes place, validating that the ACE is also prone to allogeneic reactions[21–23]. As a proof-of-concept study, we used an intravitreal dexamethasone implant to evaluate the efficacy of local immunosuppression to alleviate allograft deterioration in this novel islet transplantation site, the ACE of a NHP model.

Material and Methods

Research Design and Animals

A total of seven cynomolgus non-human primates (*Macaca fascicularis*; around 20 years old, male, 5–7 kg) were enrolled in this study. The monkeys received allogeneic pancreatic islet transplantations and were divided into two study groups, that is, the control group with no immunosuppression (n = 4 eyes; 3 monkeys) and local immunosuppression using dexamethasone implant (n = 8 eyes; 4 monkeys). All monkeys were healthy without any metabolic disorders. The animals were housed in 2-in-1 tier cages in compliance with the guidelines of National Advisory Committee for Laboratory Animal Research (NACLAR). Enrichment toys, supplementary fruits, and ad libitum water were provided throughout the experiment. Health checks and infection screenings were done bi-annually. The animals were anesthetized using intramuscular ketamine and isoflurane gas at 3% to 5% for induction and 1% to 2% for maintenance during the experiments to alleviate pain and discomfort. Vital signs such as heart rate, respiratory rate, and body temperature were monitored throughout the procedures. Pre-operative and post-operative care were provided accordingly. All the experiments were carried out in accordance with approved protocols (2018/SHS/1418) and guidelines of the Institutional Animal Care and Use Committee (IACUC) of SingHealth, Singapore.

Pancreatic Islet Isolation and Transplantation

Pancreatic islets were isolated from the whole pancreas of a healthy donor NHP procured by total pancreatectomy. The pancreas was flushed and stored in chilled UW solution until

islet isolation. The pancreas was perfused with Liberase enzyme (Roche, Basel, Switzerland) via the pancreatic duct. The pancreas was further cut into pieces and transferred into a Ricordi Chamber (Biorep Technologies, Florida, USA), where the pieces were agitated manually to facilitate the separation of islet cells from the exocrine matrix. The pancreatic islets were then purified by a non-continuous gradient separation method using Ficoll gradients (Corning, Arizona, USA). The isolated islets were cultured in supplemented CMRL media (Gibco, Thermo Fisher, Massachusetts, USA) and transduced for 24 hours with adeno-associated virus-1 (AAV-1) with cytomegalovirus (CMV) promoter-driven expression of enhanced green fluorescent protein (EGFP). Around 100 islets were incubated in 2 ml of CMRL media with 5 µl of AAV1-CMV.EGFP (Addgene, Massachusetts, USA). The transduced islets were verified under a fluorescence microscope (SP8, Leica, Germany) for EGFP fluorescence before transplantation. A small self-sealing incision was made on the clear cornea, 3 mm away from the cornea-sclera junction, and around 50 islets were transplanted into the ACE of each monkey as previously described[8]. A 0.5 ml bolus dose of sub-conjunctival dexamethasone (4 mg/ml; DBL, Pfizer, New York, USA) injection was given immediately after the transplantation. Periodic images of whole irises were taken across 24 weeks using an upright fluorescence microscope to monitor islet graft survival. Islet grafts that lost fluorescence were considered as graft rejections.

Local Application of Sustained-Release Dexamethasone Pellet

There was no systemic immunosuppression used in these experiments. A local injection of 700 µg of micronized dexamethasone pellet (Ozurdex, Allergan, Dublin, Ireland) was given via intravitreal route 3 days before islet transplantation to 4 of the monkeys (n = 8 eyes). The peri-ocular region was sterilized with half-strength povidone-iodine followed by a physiological saline wash. A self-sealing puncture wound was made 2 mm behind the limbus area using a 22G needle attached to an applicator loaded with a dexamethasone pellet. The pellet was then injected into the vitreous space at the superior portion of the eyeball. Aqueous humor from the ACE and peripheral plasma were collected at pre-determined time points and used to measure local and systemic levels of dexamethasone.

Measurement of Intraocular Pressure

The monkey was anesthetized using an intramuscular ketamine (10 mg/kg) and placed in a supine position. One drop of 0.5% Alcaine eyedrop (Alcon, Geneva, Switzerland) was applied onto the cornea. A handheld Tono-Pen AVIA Vet Veterinary Tonometer (Reichert Technologies, Depew, NY, USA) was used to measure the intraocular pressure in both eyes. A total of 6 measurements were taken for each eye at each timepoint and mean intraocular pressure was reported.

Quantification of Dexamethasone Level Locally and Systemically

The levels of dexamethasone in the aqueous humor at weeks 0, 4, 8, 12, 15, 18, 21, and 24 (local distribution) and the plasma at weeks 0, 4, 8, 12, 15, and 21 (systemic distribution) were analyzed using a high-performance liquid chromatography-tandem mass spectrometry (LC-MS/MS) Shimadzu LCMS-8040 system (Shimadzu Corp., Japan) in multiple reaction monitoring (MRM) mode. Hydrocortisone was used as an internal standard. Samples of 10 µl (both aqueous and plasma) were injected at a flow rate of 0.8 mL/min into the Gemini NX-C18 100 mm x 3 mm cartridge with a C18 security guard cartridge. The mobile phase consisted of filtered 5 mM Ammonium Acetate as solvent A and Acetonitrile as solvent B. The linear range of the test was 2.5 to 100 ng/mL. Values lower than 2.5 ng/mL were extrapolated values.

Functional Assays

For the assessment of islet graft function, C-peptide levels in the aqueous humor collected at weeks 0, 4, 8, 12, 15, 18, and 24 were determined using an ultrasensitive C-peptide ELISA kit (Mercodia, Uppsala, Sweden). To evaluate the glucose-stimulated insulin secretion of the grafts, the engrafted islets were dissected out together with a small portion of surrounding iris tissue after enucleating the eyeballs. The extracted islet grafts were incubated at 3 mM glucose followed by 11 mM glucose at 37°C, and the supernatants collected from both conditions were used to measure the amount of insulin released using an insulin ELISA kit (Mercodia, Uppsala, Sweden). Since the number of islets transplanted was small in this study, we did not measure the systemic insulin or C-peptide level as we did not expect a significant change in the circulation.

Immunofluorescence Staining of Islet Grafts

The enucleated eyeballs were fixed in 4% paraformaldehyde solution, cryo-sectioned into 10 µm thick sections and mounted on polylysine coated slides. The slides were then blocked in 4% bovine serum albumin (Sigma-Aldrich, Merck, Darmstadt, Germany) followed by overnight incubation with primary antibodies specific for insulin (Dako, Agilent, California, USA), glucagon (Santa Cruz, Texas, USA) and somatostatin (Chemicon, Merck, Darmstadt, Germany) to stain the different islet cell populations, that is, beta, alpha, and delta cells. The sections were then incubated with secondary antibodies (Alexa Fluor, Invitrogen, Thermo Fisher Scientific, Oslo, Norway) and mounted in an anti-fade

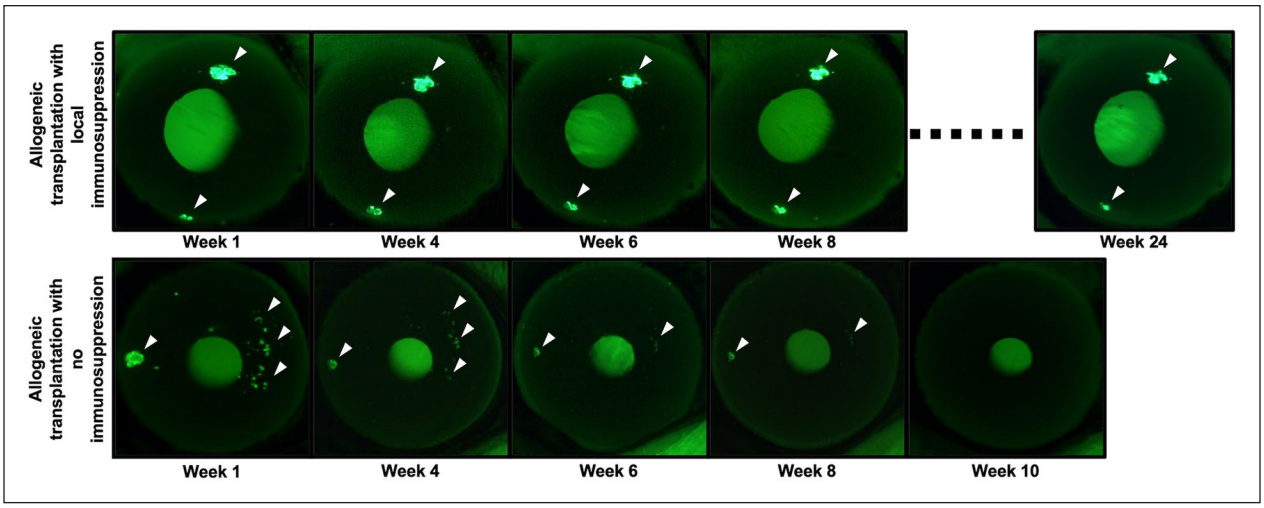

Figure 1. Survival of allogeneic pancreatic islet grafts. Green fluorescent protein expressing pancreatic islet grafts can be seen on the monkey iris. Allogeneic islet grafts survived longer with local immunosuppression treatment (up to week 24) compared to no immunosuppression (up to week 10). Islets are indicated with white arrows.

medium (ProLong Glass Antifade Mountant, Invitrogen, Thermo Fisher Scientific, Oslo, Norway). The sections were also stained with $CD4^+$ and $CD8^+$ specific antibodies (BD Biosciences, California, USA) to determine direct evidence of T-cell mediated immune destruction to the allografts.

Statistical Analysis and Graphical Illustrations

Pancreatic islet graft survival was assessed by counting the number of fluorescence positive islets on the iris. Kaplan-Meier survival curves were constructed using GraphPad Prism version 6.03 (GraphPad Software, San Diego, California, USA, www.graphpad.com) to determine the efficacy of local dexamethasone treatment on graft survival in allogeneic islet transplantation. A log-rank test was calculated on the Kaplan-Meier survival curves, and a *P*-value of less than 0.05 was considered significant. Curves for graft survival, C-peptide levels in the aqueous humor, glucose-stimulated insulin secretion (GSIS), dexamethasone levels in the aqueous humor and plasma were generated using GraphPad Prism software.

Results

Pancreatic Islet Transplantation

After 24 hours post-transduction with the CMV-EGFP encoding AAV-1 vector, the transduced islets showed green fluorescence when excited with 488 nm light. In a few cases, islets aggregated with each other and resembled big islets on the irises of some of the monkeys. The transplanted islets engrafted well, and apparent revascularisation was seen from 3 weeks after transplantation (Supplemental Fig. S1). Fluorescence from the engrafted islets was observed on the irises of the monkeys and was used to determine graft survival (Fig. 1). No complications such as uveitis, cataract formation, endophthalmitis, or infection were noted in the transplanted eyes. We found that 2 eyes out of 8 eyes treated with dexamethasone implant showed a transient rise of IOP to 20 to 25 mmHg from week 8 to week 18, which subsequently dropped back to less than 20 mmHg (Supplemental Fig. S2). It was expected as a long-term dexamethasone implant was shown to be associated with increased intraocular pressure[24,25]. No IOP lowering agent was administered to avoid influence on the experimental outcomes.

Islets Survived for Up to 6 Months After a Single Dose of Dexamethasone Treatment

After transplantation, islet survival was assessed at predetermined time points (1, 2, 3, 4, 6, 8, 10, 12, 15, 18, 21, and 24 weeks) (Fig. 2A). At week 3, 37.5% of the allogeneic islets without immunosuppression treatment survived, whereas the islets treated with local dexamethasone showed no deterioration. Without immunosuppression, only 8.3% of the islet grafts were accounted for at week 8, followed by complete destruction by week 10. On the other hand, it was observed that up to week 5, only a minor destruction of the islet grafts (95% survival) occurred in the local dexamethasone treated group. Around 73% of the treated islets were observed at week 10, where in contrast, the control group showed zero survival. Noteworthy, allogeneic islets transplanted under conditions with local dexamethasone treatment showed a significantly better survival (median survival time- 15 weeks vs 3 weeks, log-rank test $P < .0001$) compared to allogeneic control islets without any immunosuppression (Fig. 2B). With a single dexamethasone pellet

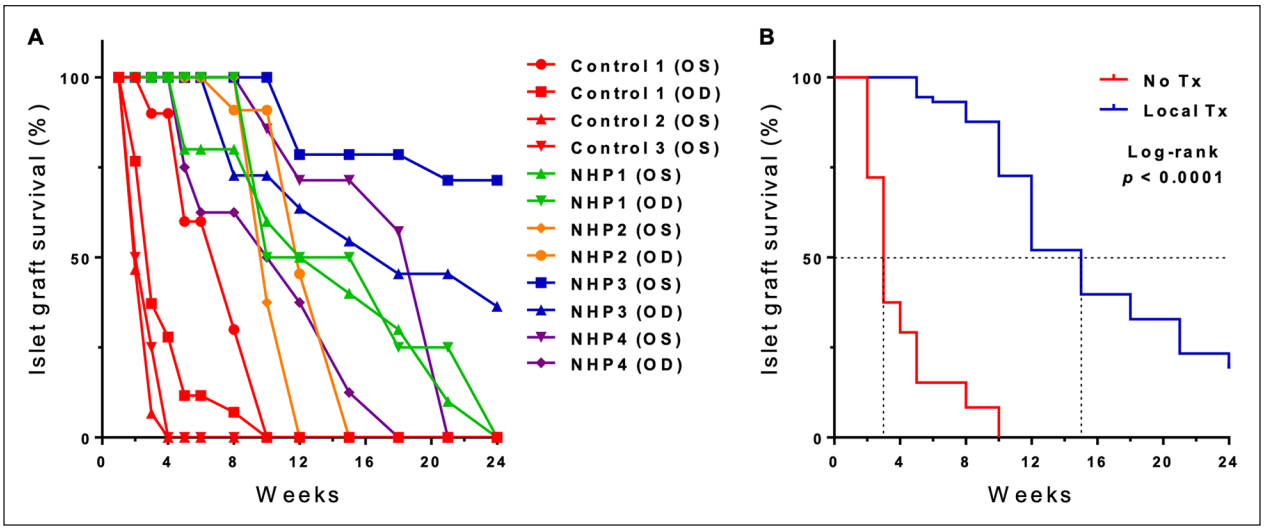

Figure 2. Pancreatic islet graft survival curves. (A) Islet graft survival in the individual eyes. Islet grafts in the anterior chamber of the eye without immunosuppression treatment (Control) deteriorated faster than grafts with local dexamethasone treatment. (B) Kaplan-Meier survival curves of the allogeneic islet grafts (local dexamethasone treatment, blue, n=8 eyes vs no treatment, red, n=4 eyes); Graft survival is significantly longer in the local dexamethasone-treated group (log-rank, P<0.0001), compared to no immunosuppression treatment (median survival time - 15 weeks vs 3 weeks). OS: Oculus Sinister, left eye; OD: Oculus Dexter, right eye.

injection, around 20% of the treated islet grafts remained at 24 weeks after transplantation.

Dexamethasone Was Present in the Aqueous Humor and Plasma

Dexamethasone was detected in the aqueous humor at week 4 (2.57 ± 0.67 to 13.63 ± 0.99 ng/mL), with a peak concentration at week 8 (15.55 ± 2.19 to 89.18 ± 9.65 ng/mL) and started to decline from week 12 (0.92 ± 0.3 to 10.31 ± 0.17 ng/mL). Dexamethasone was no longer detected from week 15 onwards in the aqueous humor. A small amount of dexamethasone was detected in the plasma (1.28 ± 0.48 to 3.3 ± 0.46 ng/mL) at week 8 in all monkeys that received local dexamethasone implants. One monkey (NHP3) demonstrated a sustained level of dexamethasone (1.24 ± 0.27 ng/mL) in the systemic circulation at week 12 (Fig. 3).

Intraocular Islets Were Functional With Local Dexamethasone Treatment

A dynamic pattern of C-peptide levels was detected in the aqueous humor throughout the experimental period, with a transient dive between weeks 8 and 12. This same period coincided with the highest dexamethasone level detected in the aqueous humor, suggesting that dexamethasone may have a transient and reversible suppressive effect on insulin secretion. C-peptide levels recovered from week 12 onwards (Fig. 4A). The GSIS assay showed a good insulin secretion index of 3.04 ± 0.33 in response to high glucose stimulation in 2 irises with islet grafts surviving at week 24 (Fig. 4B).

Local Dexamethasone Delayed T-Cells Mediated Graft Destruction

All components of the islet, that is, alpha, beta, and delta cells, were still present in the islets from NHP3 OS (Oculus Sinister, left eye) that were surviving at the end of the experiment at week 24. The islets that died off just before the experimental endpoint (NHP1 OS) showed remaining alpha cells and delta cells, suggesting that the beta cells were more sensitive to the immune reactions (Fig. 5A). Immunostaining of the grafts showed that there was focal involvement of $CD4^+$/$CD8^+$ (46%/54%) T-cells in the islets that were still surviving at week 24 (NHP3 OS, Fig. 5B), suggesting that local dexamethasone treatment delayed T-cell infiltration into the islet grafts, a key process of allogeneic immune destruction.

Discussion

Our findings emphasize the feasibility of confined anti-inflammatory or immunosuppression treatment for the preservation of pancreatic islet allografts in the ACE of a NHP model, a surrogate for humans. Allogeneic islet grafts in the dexamethasone milieu survived significantly longer than those in the absence of immunosuppression. Immune specific staining of the grafts showed that local treatment with dexamethasone delayed T-cell mediated destruction of the allogeneic islet grafts (Fig. 5B).

Local anti-inflammatory or immunosuppression treatment is an attractive alternative approach to a systemic route in organ transplantation, as the latter involves several complications and causes undesirable long-term side effects in

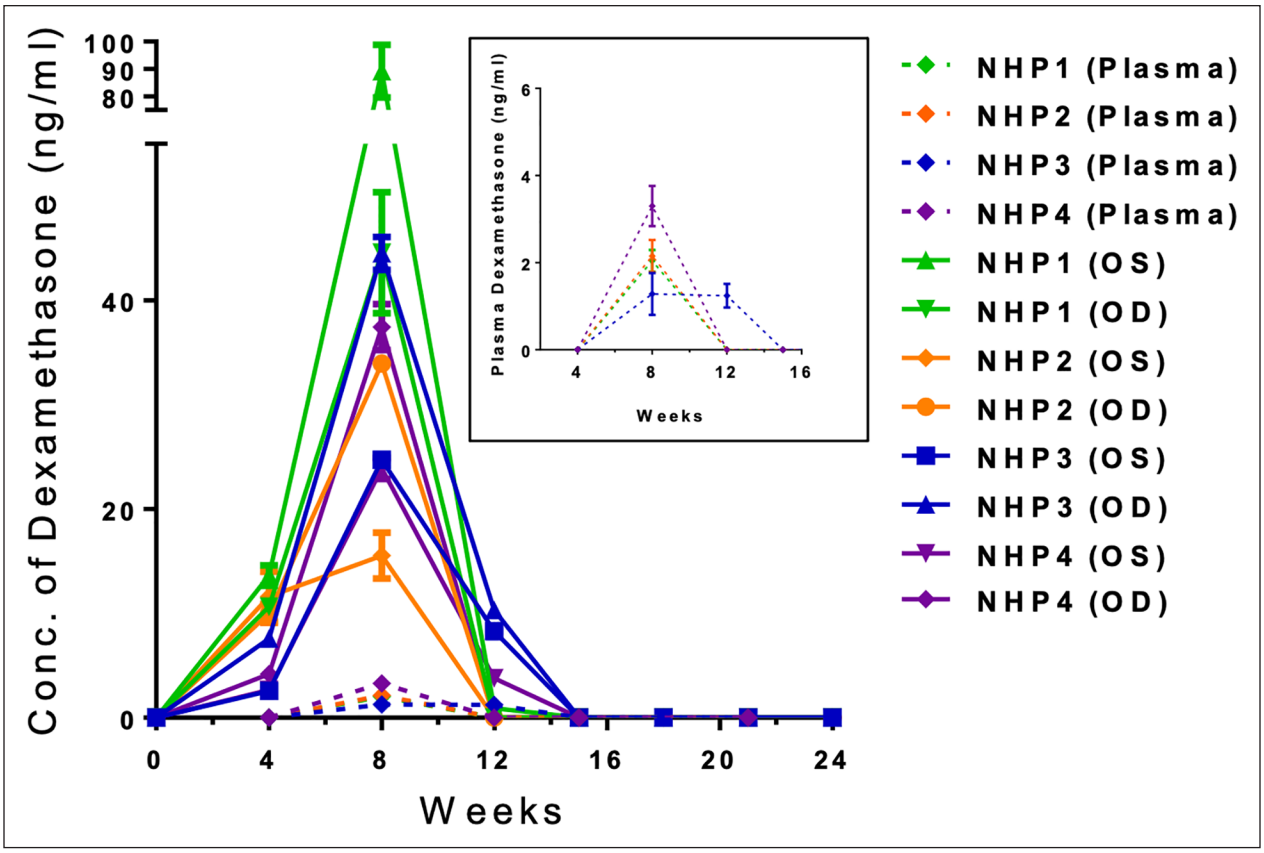

Figure 3. Dexamethasone level in the aqueous humor and plasma. Dexamethasone level was highest at week 8 in both aqueous and plasma (Values are in means ± SD). The inset shows dexamethasone level in the plasma of each monkey. OS: Oculus Sinister, left eye; OD: Oculus Dexter, right eye.

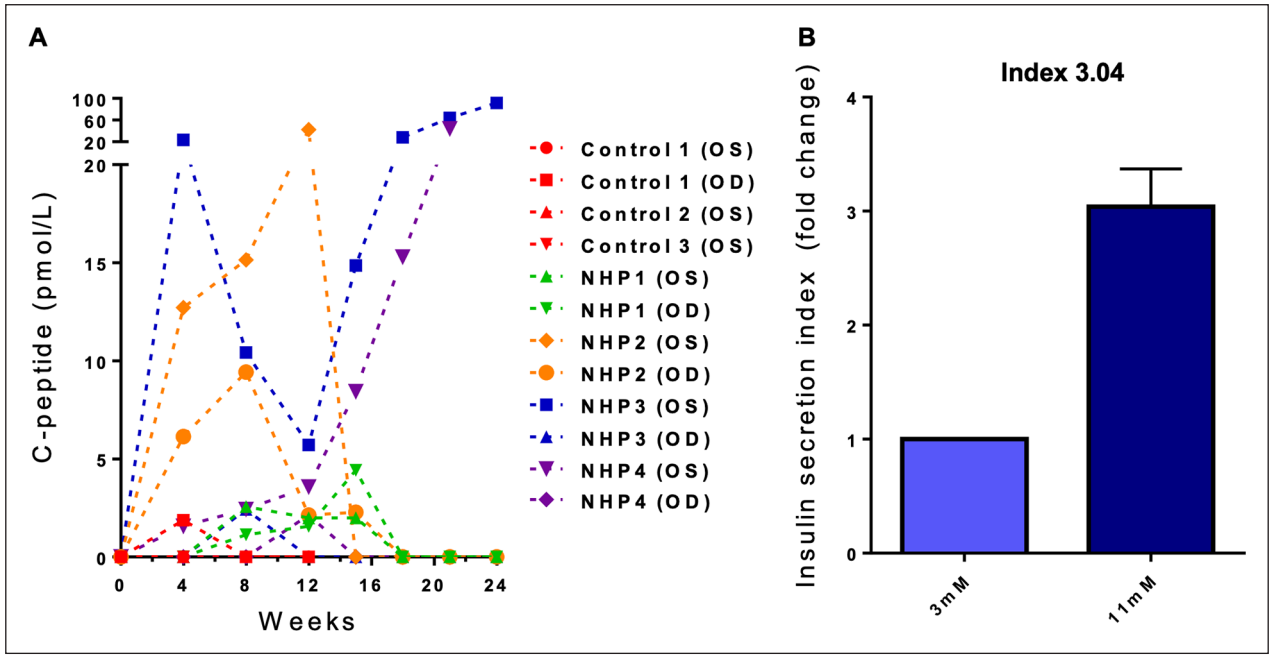

Figure 4. Functional assays of islet grafts. (A) C-peptide levels in the aqueous humor of individual eyes. (B) GSIS (Glucose stimulated insulin secretion assay). Islets were incubated at 3 mM and 11 mM glucose solution to measure insulin secretion in response to glucose stimulation. There was an insulin secretion index of 3.04. Insulin response showed in 2 out of 2 irises surviving till end of the experiment (Values are in means ± SD). OS: Oculus Sinister, left eye; OD: Oculus Dexter, right eye.

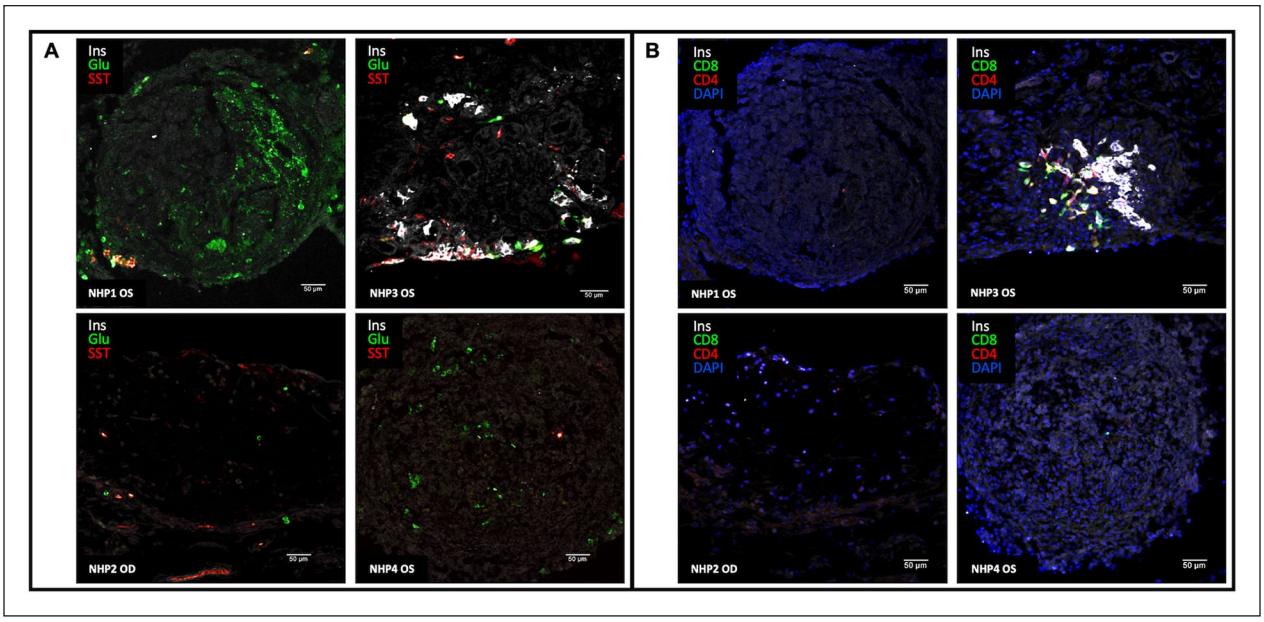

Figure 5. Immunostaining of islet grafts. (A) Immunostaining of Ins (beta cells), Glu (alpha cells) and SST (delta cells) was performed on the islet grafts. All pancreatic islet cells are present in NHP3 OS (The scale bars are 50 μm). (B) Immunostaining specifically of $CD4^+$ and $CD8^+$ cells were done on the islet grafts. There was a delayed $CD4^+$ and $CD8^+$ T-cells involvement in the graft in NHP3 OS (The scale bars are 50 μm). Ins: insulin; Glu: glucagon; SST: somatostatin; OS: Oculus Sinister, left eye; OD: Oculus Dexter, right eye.

patients[13]. However, the feasibility of local immunosuppression treatment depends on the nature of the transplantation site. The traditional site for islet transplantation, namely the portal vein of the liver, is not feasible for local confined immunosuppression treatment. Alternative sites feasible for confined local immunosuppression are subcutaneous space, epididymal fat pad, omentum, and anterior chamber of the eye. Skin and subcutaneous space is attractive for its potential of accessibility for re-transplantation and routine graft biopsy. Nonetheless, because of its hypo-vascularity and poor oxygenation in nature, creation of pre-vascularised space for the islets is needed to improve graft survival[26–31]. By encapsulating the islets in biocompatible immunomodulatory materials such as basic fibroblast growth factor agarose rods and Islet Viability Matrix (IVM), the islet grafts showed better survival in the subcutaneous space and the recipients achieved euglycemia[32,33]. On the other hand, due to its large space for islets, abundant blood supply with portal venous drainage, omentum is considered as one of the ideal alternative transplant sites for the pancreatic islets[34–37]. By using biological scaffolds, encapsulation with semi-permeable hydrogel and biocompatible matrix locally improved the survival of the islet grafts[38–42]. In a recent clinical trial, Baidal et al[43] reported that islet transplantation on the omentum in a T1DM patient, achieved normoglycemia and insulin independence for 12 months. However, the challenges include laparotomy and its complications, inability to re-introduce more islets and necessity of special encapsulation techniques to improve the islet grafts survival.

Because of its oxygen rich milieu, immune privilege, serving as a natural confined incubator for the islets and minimally invasive surgical approach, the ACE is an attractive site for islet transplantation with possibility to locally modulate immune responses. Islet transplantation into the ACE is not associated with instant blood mediated inflammatory reaction (IBMIR), and thus immediate islet cell loss is dramatically reduced. In addition, because of its immune privilege nature of the ACE, the newly transplanted islets are also spared from proinflammatory cytokine reactions. The intraocular islet grafts also showed revascularisation and innervation which mimic the endogenous islets[19,44–46]. The major advantage of the ACE as a transplantation site is the possibility to employ several routes to deliver and administer repeated applications of medication or immunosuppressive compound locally to improve the survival and function of the intraocular islet grafts[47–50].

In our experiments, we applied micronized dexamethasone pellet locally to the eyes 3 days before intraocular allogeneic islet transplantation. This pellet allows a sustained release of dexamethasone, a potent glucocorticoid that inhibits proliferation and differentiation of both $CD4^+$ and $CD8^+$ T-cells by attenuating the CD80 mediated and CD28 co-stimulatory pathways by upregulating CTLA-4[51]. In addition, corticosteroids suppress the expression of cytokines, chemokines and inflammatory proteins and upregulate anti-inflammatory genes such as annexin-1, SLPI, Interleukin-10, and NF-κB inhibitor[52]. Our experiments demonstrated a significant delay in T-cell-mediated destruction of the islet grafts in the ACE

following local dexamethasone administration. In pre-clinical studies of NHPs, it was shown that dexamethasone released from the Ozurdex pellet could last for up to 6 months in the retina and vitreous after implantation[53]. We found that dexamethasone was present in the ACE for 3 months, with a peak at 2 months. The observation of a shorter duration of dexamethasone in the ACE is likely due to a vigorous clearance nature of aqueous fluid. Nevertheless, our data suggest that a single dexamethasone pellet can preserve the majority of islet grafts for 10 weeks (73% survival). Hence, we hypothesize that the efficacy of the Ozurdex pellet for intraocular islet graft preservation is around 10 weeks, and booster applications should be applied at such an interval. Nonetheless, side effects of Ozurdex administration should be considered which include conjunctival hemorrhage, ocular discomfort, intraocular pressure elevation, cataract formation, and not commonly macula hole formation[24,25,54–56]. Notably, long-term investigation of Ozurdex usage showed no significant adverse reaction[57].

Dexamethasone has a direct effect on insulin secretion by binding to the glucocorticoid receptors in the beta cells of the pancreatic islets. It was shown that dexamethasone affects beta cell Ca^{2+} handling, which in turn inhibits insulin secretion in a reversible manner[58,59]. Inhibition of insulin secretion by dexamethasone was shown to be dose-dependent, where a low dose of 20 nM (8 ng/mL) suppressed insulin secretion by 50% and 250 nM (100 ng/mL) reduced it up to 75%[59] . It was also shown that 1 to 2.5 mg/mL of dexamethasone scaffolds could significantly improve islet engraftment and glucose homeostasis, where a higher dose of 5-10 mg/mL showed an unfavorable effect on engraftment[16]. In our experiments, and consistent with the above findings, dexamethasone distributed in the aqueous humor showed a short-term suppression of insulin secretion in a reversible manner with an observable beneficial effect on islet graft survival. Our data suggest that less than 100 ng/mL of dexamethasone substantially improved intraocular islet graft survival. We also noted that the graft survived longer in the monkey (NHP3) with an extended period of systemic dexamethasone presence, suggesting that a modest amount of systemic dexamethasone further improved the preservation of allogeneic islet grafts. Notably, the natural aqueous drainage system of the ACE prevents the accumulation of dexamethasone over time, allowing a favorable milieu for the engrafted islets.

Interestingly, inhibition of insulin release by dexamethasone can be attenuated by raising cyclic-AMP (cAMP)[59]. This opens new considerations and future aspects applying the ACE model, enabling local administration of therapeutic drugs to the eye. Administering adrenergic agents, i.e. Epinephrine, Phenylephrine and Forskolin, to the eye have been shown to reduce IOP by increasing cAMP concentration in the aqueous humor, trabecular meshwork cells and ciliary processes which in turn controls aqueous humor formation and dynamics[60–62]. Such eye drops may be used together with the dexamethasone treatment to subvert dexamethasone-induced impairment of insulin secretion.

Notably, no tolerance was observed in grafts with 12 weeks of exposure to dexamethasone, suggesting that a longer incubation time may be necessary to induce tolerance. In our previous experiments, where a baboon received systemic transient immunosuppression treatment using anti-CD154 (CD40L) antibody for 248 days, showed that the islet grafts in the ACE were able to survive for more than 400 days after suspending immunosuppression treatment. It was also shown that islet allografts in the ACE were persevered for more than 300 days in mice with a transient systemic treatment with anti-CD154 antibody during the peri-transplant period (days -3, -1, 0, 3 and 7; day 0 = islet transplantation)[11]. Hence, as a treatment strategy, it may be beneficial to apply brief systemic immunosuppression during the peri-transplantation period followed by a local immunosuppression therapy. Although we did not study the inflammatory profile in detail due to sample volume limitation, it should be considered for future experiments.

Our data suggest that local immunosuppression significantly improves the survival of allogeneic islet grafts in the ACE. It bears significant implication on clinical islet transplantation as the feasibility of using local immunosuppression may drastically reduce the negative side effects of systemic immunosuppression and invasive transplant procedures. It can tilt the risk-benefit ratio, allowing a larger population to be eligible for islet transplantation to manage diabetes. In conclusion, the ACE opens up novel and versatile possibilities for efficient local administration of pharmacological compounds, enabling improvement in function and survival of pancreatic islet grafts in the context of diabetes.

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Author Contributions

P-O.B. is the originator of the idea of transplanting pancreatic islets into the ACE of monkeys. S.B.B.T., V.A.B. and P-O.B. designed the experiments. S.B.B.T., M.C., V.A.B. and G.T.S.W. conducted the experiments. S.B.B.T., I.L. and P-O.B. analyzed the results. I.L. and Y.A. advised on the experimental design. S.B.B.T. drafted the manuscript. All the authors commented upon, critically revised, and approved the submission of the manuscript. P-O.B. is the guarantor of this work and, as such, has full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Abbreviations

AAV-1, Adeno associated Virus-1; ACE, Anterior Chamber of the Eye; CMV, Cytomegalovirus; EGFP, Enhanced Green Fluorescent Protein; IBMIR, Instant Blood Mediated Inflammatory Reaction;

IOP, Intra-ocular Pressure; NHP, Non-Human Primates; OS, Oculus Sinister (left eye); OD, Oculus Dexter (right eye); POD, Post-Operation Day; T1DM, Type 1 Diabetes Mellitus; T2DM, Type 2 Diabetes Mellitus.

Data and Resource Availability

The data sets generated from the current study are available from the corresponding authors upon request.

Declaration of Conflicting Interest

The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: P-O.B. is a co-founder and CEO of Biocrine, a small Biotech Company that uses the ACE as a tool in diabetes research and clinic, and I.L. is a consultant for the same company.

Ethical Approval

Ethical Approval is not applicable for this article.

Statement of Human and Animal Rights

All procedures in this study were conducted in accordance with the Institutional Animal Care and Use Committee (IACUC), SingHealth, Singapore (2018/SHS/1418).

Statement of Informed Consent

There are no human subjects in this article, and informed consent is not applicable.

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Supplemental Material

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