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Impact of Oxygen on Pancreatic Islet Survival

Hirotake Komatsu, MD, PhD, Fouad Kandeel, MD, PhD, and Yoko Mullen, MD, PhD

Abstract: Pancreatic islet transplantation is a promising treatment option for individuals with type 1 diabetes; however, maintaining islet function after transplantation remains a large challenge. Multiple factors, including hypoxia associated events, trigger pretransplant and posttransplant loss of islet function. In fact, islets are easily damaged in hypoxic conditions before transplantation including the preparation steps of pancreas procurement, islet isolation, and culture. Furthermore, after transplantation, islets are also exposed to the hypoxic environment of the transplant site until they are vascularized and engrafted. Because islets are exposed to such drastic environmental changes, protective measures are important to maintain islet viability and function. Many studies have demonstrated that the prevention of hypoxia contributes to maintaining islet quality. In this review, we summarize the latest oxygen-related islet physiology, including computational simulation. Furthermore, we review recent advances in oxygen-associated treatment options used as part of the transplant process, including up-to-date oxygen generating biomaterials as well as a classical oxygen inhalation therapy.

Key Words: islet transplantation, type 1 diabetes, hypoxia, oxygen, hyperoxia

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In 1990, Scharp et al from Washington University in St. Louis first demonstrated that islet transplantation could free an individual with type 1 diabetes (T1D) from insulin injections.[1] This achievement was followed by successful islet transplantations in 9 diabetic patients after upper abdominal exenteration and liver transplantation, carried out at University of Pittsburgh and University of Miami.[2] Although both pancreatic digestion and islet isolation methods have advanced since the 1990s, it has taken nearly 10 additional years before islet transplantation was recognized as an alternate to exogenous insulin injections for T1D patients. In 2000, Shapiro et al from University of Alberta introduced the Edmonton Protocol, an islet alone transplantation method and glucocorticoid-free regimen for immunosuppression.[3] This protocol represents a milestone toward the adoption of islet transplantation, and today islet transplantation promises to free T1D patients from reoccurring insulin injections and the lethal adverse events of hypoglycemia. However, the insulin independence rate gradually declines with time—50% insulin independence at one year after completion of islet transplantation decreases to approximately 25% at 5 years.[4,5]

Investigators, including ourselves, believe that the hostile environment of the liver, the currently preferred islet transplantation site, contributes to chronic islet damage and loss. This has led to a search for sites outside of the liver for islet transplantation, and the results of these accumulated studies have uncovered the importance of oxygen (O_2) for islet survival regardless of the islet transplantation site.[6–9] In this review, we provide an overview of the latest knowledge of O_2-related islet physiology and of methods to supply sufficient O_2 for islet transplantation.

UNDERSTANDING THE O_2 MICROENVIRONMENT EXPERIENCED BY ISLETS

Oxygen Microenvironment of Native Islets in the Pancreas

Earlier studies revealed that islets receive 10% to 15% of arterial blood despite occupying only 1% of the total pancreas mass.[10,11] This indicates that the amount of O_2 per islet volume that is transported by hemoglobin (Hb) is greater than that of other pancreatic parenchyma. Thus, O_2 is readily available to satisfy the metabolic demands of native islet cells and to maintain their physiological activities.[12] However, the availability of a large amount of O_2 to any specific tissue does not necessarily indicate that the tissue has high partial O_2 tension (pO_2). Rather, pO_2 depends on the type of blood supply to the tissue and the density of the microvessels. For example, the blood supply to the liver comes largely from the portal vein, rather than the hepatic artery. Thus, the pO_2 of liver tissue is similar to that of the portal vein. On the other hand, the pancreas receives its blood supply from the artery. Thus, the pO_2 of pancreatic tissue is higher than that of the liver. This explanation is justifiable especially when the tissue is well irrigated by the vessels and O_2 is uniformly distributed. Namely, the density of microvessels also affects the tissue pO_2 because poorly vascularized tissue causes an imbalance between O_2 supply and O_2 consumption thereby creating an O_2 gradient (Fig. 1A). Oxygen imbalance and gradient occurs in a specific area in the brain exposed to systemic hypotension; distal field between 2 main arterial territories results in the O_2 depletion causing so-called watershed infarction.[13,14] A similar phenomenon takes place inside tiny isolated islets in culture or in islets after transplantation. This is caused by the loss of a supply of blood provided through the vascular network.

Oxygen Microenvironment of Isolated Islets

Before transplantation, islets are isolated from a donor pancreas through enzymatic and mechanical digestion. This isolation process disconnects islets from the surrounding tissues, including

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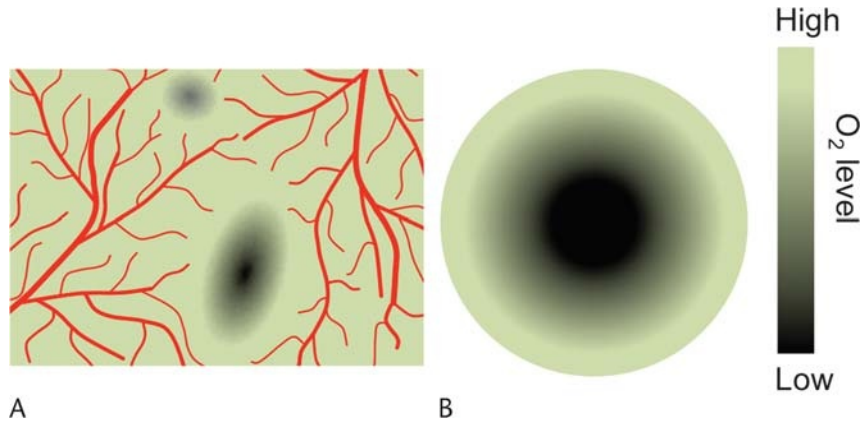

FIGURE 1. Oxygen gradient in tissue and isolated islets. Schematics of O_2 gradient. A, The low vessel density causes imbalance between O_2 supply and O_2 consumption in tissue. Oxygen supply relies on diffusion, which creates an O_2 gradient and induces low O_2 concentration (gray). B, Illustration of oxygen gradient inside an idealized spherical islet structure. Isolated islets are disconnected from donor blood vessels; therefore, O_2 diffusion from the surface is the only supply of O_2. Because the islet cells consume O_2, an O_2 gradient is generated toward the core.

blood vessels. Even without a vascular connection, isolated islets can survive in vitro or in vivo if O_2 is available through diffusion from the surrounding environment, such as culture medium or well-oxygenated tissue after transplantation. Oxygen that permeates through the islet surface is used by cells in the mantle, which forms an O_2 gradient within the islet structure (Fig. 1B). Metabolically active endocrine cells in the islets consume more O_2, thus, the pO_2 level rapidly drops near these cells. Other factors influencing pO_2 inside the islets are O_2 diffusivity, O_2 solubility, the Michaelis O_2 constant, and islet diameter.[15–17] Among those factors, the islet diameter and ambient O_2 tension are variables that influence the pO_2 inside of the islet. This is illustrated by the fact that larger islets frequently exhibit central necrosis, which is speculated as a result of an insufficient O_2 supply. However, an assessment of O_2 gradient and hypoxia inside of the islet structure is difficult because its small structure makes quantitative measurements difficult.

Oxygen Distribution Inside Isolated Islets

To prove that the hypoxia is the primary cause of the central necrosis of cultured islets, we used computational simulations to reveal the O_2 gradient inside the islet structure. The results showed markedly low pO_2 in the islet core. To correlate low pO_2 to cell necrosis, we also assessed the viability of 1278 cultured human islets to measure the dead cell volume. This dead cell volume was then compared with the volume of islet hypoxic area obtained by in silico simulation data.[18] Our results demonstrated that the hypoxic volume calculated in silico was in direct proportion to dead cell volume measured in vitro in the islets. In silico simulation was also utilized by other investigators to demonstrate the detrimental effect of thrombus formation on the surface of islets following intraportal transplantation.[17] The thrombus formation increased anoxic volume fraction (14% without thrombus to 30%) and functional loss (72% without thrombus to 90%), indicating that pO_2 was greatly reduced inside the islet by formation of a mantle-shaped structure around the islet, which increased islet diameter and interfered with O_2 diffusion. In silico simulations were also used to simulate the pO_2 of culture medium surrounding islets and showed that an O_2 gradient formed in media in culture flasks.[15] The O_2 level was highest at the surface and lowest at the bottom of the flask where the cells are cultured. These studies demonstrated that in silico simulation plays an important role in uncovering islet physiology.

Consistent with in silico simulations, larger islets develop a hypoxic core and are more likely to undergo cell death. This implies that smaller islets may achieve better islet survival, engraftment, and transplantation outcome as suggested in previous studies.[19–21] These studies also showed better insulin secretion of smaller islets in vitro in culture. In clinical studies, the analysis of the islet size index (the number of islet equivalent [IEQ] divided by the number of islet particles) of transplanted islets and the posttransplant graft function as measured by the stimulated C-peptide response also demonstrated the advantage of smaller islets to achieve better islet transplantation results.[19] Furthermore, the size index of the islets is also found as a useful predictor of achieving insulin independence in islet autotransplantation.[20]

EFFECT OF OXYGEN ON ISOLATED ISLETS

In the following section, we discuss the effects of O_2 on isolated islets. In general, normoxia is defined as the O_2 environment containing 21% O_2 (same as ambient air). However, normoxia may not be a "normal" or "physiological" microenvironment suitable for survival and physiological function of isolated islets. Rather, "normoxia" conditions may provide an insufficient O_2 supply to isolated islets that leads to hypoxia.

Effect of Hypoxia on Isolated Islets

Anemia reduces O_2 delivery throughout the body due to lower Hb levels. Chronic anemia was reported to lead to deteriorated β-cell function.[22] In fetal sheep, both acute and chronic anemia induce elevated blood glucose levels and β-cell dysfunction as measured by glucose tolerance tests (GTTs) in vivo. Intriguingly, islets isolated from anemic and normal sheep did not show any functional difference in vitro, indicating that islet dysfunction is reversible. The arterial pO_2 of anemic sheep was maintained similar to that of the control sheep (89.0 vs 92.9 mmHg, respectively), indicating that O_2 to native islets in the anemic sheep was supported through diffusion. Elevated blood glucose in the anemic sheep suggested that O_2 was not sufficient to support β-cell functionally because of the shortage of circulating Hb. In contrast, the exposure of neonatal rats to intermittent hypoxia in

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a second study resulted in irreversible impairment of β-cell function.[23] Neonatal rats inhaled 10.3% and 20.8% O_2 alternately every 7 minutes for 5 hours. During this experimental period, the blood pO_2 was assumed to be lower than 50 mmHg with 10.3% O_2 inhalation, whereas it was maintained at 90 to 100 mmHg of pO_2 with 20.8% O_2 inhalation. The intermittent hypoxia-treated rats showed higher fasting blood glucose and lower C-peptide levels even 3 weeks after hypoxia exposure. These results imply that (1) O_2 supply via Hb is required to maintain in vivo islet function, and (2) the decreased blood pO_2 is lethal for islets and leads to irreversible β-cell dysfunction. In another study using a similar experimental setup, rats were exposed to intermittent hypoxia through 10% and 20.5% O_2 inhalation every 4 minutes in a span of 1 hour.[24] This treatment also induced islet dysfunction as shown by the significantly decreased insulin production, whereas levels of insulin mRNA and C-peptide/insulin protein remained same as the control. The reduced insulin production was associated with the disturbance of insulin crystal precipitation due to a lack of zinc caused by transient hypoxia.

Hypoxic conditions can be induced in vitro in culture by seeding islets in a high density. In the culture vessels, O_2 is supplied from the surface of culture medium, and O_2 tension becomes lower toward the bottom of the vessels where the islets are sitting on.[15,25] In a study using rat isolated islets with different seeding densities (150–600 IEQ/cm^2), a marked decrease of pO_2 in the culture media was demonstrated in 21% O_2 for 24 hours (121.6 mmHg in 150 IEQ/cm^2 vs 80.5 mmHg in 600 IEQ/cm^2).[26] Islets were also cultured in a hypoxic condition in this study. The result showed the relations between islet viability and medium pO_2. The islet viability reduced to 95%, 85%, 75%, 55%, 45%, and 25% in 121.6, 99.5, 80.5, 14.3, 10.5, and 7.6 mmHg of medium pO_2, respectively. Similar results were obtained by culturing human islets, which showed an inverse correlation between islet viability and seeding density ranging from 75 to 600 IEQ/cm^2.[27] β-cell function, as examined by glucose-stimulated insulin secretion (GSIS) in vitro, was also impaired as the seeding density increased. Furthermore, inflammatory responses were detected in hypoxic conditions. Gene expressions of COX-1 and IL-6 in the cultured islets were upregulated,[26] and release of MCP-1 was increased.[27]

Mechanisms of impaired β-cell function by hypoxia are shown as the metabolic transition to anaerobic glycolysis. Lactate, a classic marker of hypoxia, was elevated in islets and culture media after a 24-hour hypoxic condition of 3% O_2, concomitant with the reduced ability to respond to high glucose.[28] Molecular expression changes in the islets exposed to hypoxic conditions was shown in hypoxia-inducible factor (HIF)–related pathways.[29–32] In addition, recent studies suggested the involvement of other mechanisms independent of HIF-1, by showing that the expression of genes associated with β-cell transcription pathways and insulin secretion pathways are altered after exposing isolated mouse islets and mouse β-cell line (MIN6) to hypoxia (<5% O_2) for 16 to 30 hours.[33] In these cultured cells, the expression of *Mafa*, *Pdx1*, *Slc2a2*, *Ndufa5*, *Kcnj11*, *Ins1*, *Wfs1*, *Foxa2*, and *Neurod1* genes were suppressed independent of the HIF-1 change. Another study, which also exposed mouse islets and MIN6 cells to hypoxia (1%–5% for 4–24 hours), showed the downregulation of adaptive unfolded protein response genes critical for endoplasmic reticulum (ER) homeostasis.[34] These genes are also independent from the HIF-1 pathway but related to JNK and p38 MAPK pathways. In addition, ZIP8 transporter, known as a zinc uptake transporter in β cells, was down-regulated by hypoxia exposure both in vitro and in vivo, which may be a cause of the hypoxia-induced islet dysfunction as mentioned above.[24] Calcium influx into the islet cells was also shown to induce cell damage in rat and human islets cultured in 0.8% O_2 for 5.5 hours.[35] In this regard, preventing calcium influx is a potential therapeutic target, and in fact, the potassium channel activator (diazoxide) and calcium channel blocker (nifedipine) assist the recovery of hypoxia-induced proinsulin biosynthesis and islet cell necrosis when used as preconditioning agents.

Effect of Hyperoxia on Isolated Islets

Unlike the effects of the hypoxia, the effects of hyperoxia (>160 mmHg) on isolated islets have not been well studied. Understanding islet reaction to hyperoxia is important because additional O_2 could be used to improve islet survival. Only a few studies have investigated whether hyperoxia improves islet function and sought to determine the optimal pO_2 for islet survival. Islets survive and function best in the pancreas where sufficient O_2 is available to satisfy their physiological demands. After isolation, islets are cultured under air plus 5% CO_2, which provides 21% O_2 or 160 mmHg pO_2 in culture media. This pO_2 level is set without considering the O_2 consumption by islet cells. Interestingly, it is far higher than the tissue pO_2 in the native pancreas.[36] However, this culture condition still induces "central necrosis" in the core of larger islets. Our recent studies have demonstrated that hyperoxic cultures (35% and 50% O_2) reduce the incidence and size of islet central necrosis by maintaining higher islet core pO_2.[18,25] However, if the isolated islets are cultured in a hyperoxic condition above toxic levels, a considerably large volume of cells on the islet surface are damaged (Fig. 2). Therefore, O_2 toxicity should be taken into account when islets are treated with hyperoxic conditions.

Exposure of an excised mouse pancreas to extremely high O_2 (pO_2 = 1300 mmHg achieved by a hyperbaric chamber and 630 mmHg) was highly toxic and destroyed all islet tissue in 7 days.[37] Rat islets cultured for 18 hours in 95% O_2, under the estimated pO_2 of greater than 700 mmHg, decreased β-cell GSIS function by 47% of that of the control cultured in normoxic condition.[38] In the same experimental condition, a 40% decrease of O_2 consumption rate elevated reactive oxygen species (ROS) production and increased apoptosis. The O_2 toxicity demonstrated in islets is consistent with results observed in lung cell injury in vivo by normobaric 100% O_2 exposure for 24 hours,[39–41] although the threshold of the O_2 toxicity may be cell-type specific. We recently attempted to assess the high toxicity threshold of O_2 using isolated human islets.[18] Islet viability was measured after exposing small islets to 1%, 10%, 21%, 50%, 75%, and 95% O_2 for 3 days in culture. Islets exposed to 21% and 50% O_2 (actual medium pO_2 of 160 and 270 mmHg, respectively) showed the highest viability among the conditions tested, whereas 75% and 95% O_2 (550 and 700 mmHg, respectively) significantly reduced viability. The viability of islets exposed to 95% O_2 was far lower than that cultured in hypoxia with 1% O_2. We selected only small islets to assess O_2 toxicity in this study because our in silico simulation results have shown that O_2 is uniformly distributed and that the O_2 gradient is negligible in small islets (<50 μm in diameter). Collectively, we concluded that the exposure from 21% to 50% O_2 in culture maintains islet cell viability and function best, whereas the exposure of islets above 75% O_2 (>500 mmHg pO_2) is toxic.

Our study also revealed the preferable effect of moderate ranges of hyperoxia for islets culture.[18,25] Human islets cultured under 35% to 50% O_2 maintained volume, function, and viability for 6 days similar to values measured before starting the culture. Better islet viability in these O_2 conditions was correlated with reduced central necrosis, especially in larger islets (>250 μm in diameter), indicating the contribution of higher pO_2 for prevention of hypoxia in the core of isolated islets. This study also measured the O_2 tension in culture media surrounding islets. We found that 270 and 350 mmHg of medium pO_2 corresponded to 35% and

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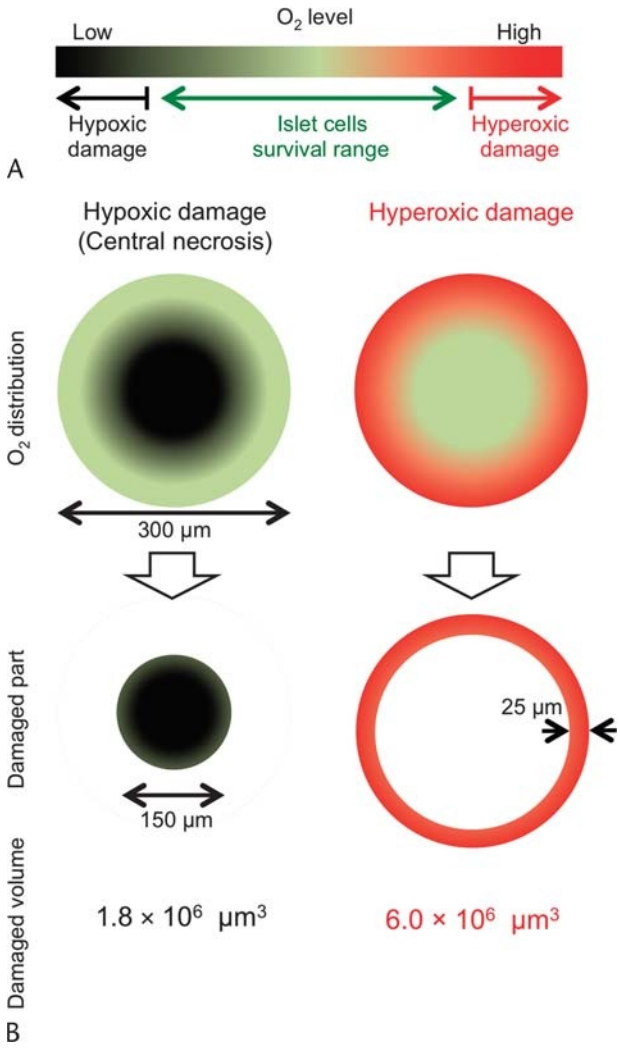

FIGURE 2. Comparison between hypoxic damage and hyperoxic damage to isolated islets. A, Islet cells survive over a specific range of O_2 levels. Cells are damaged when placed in an O_2 environment out of the viable range. B, Hypoxia causes cell damage by O_2 depletion in the islet core. In contrast, hyperoxia induces cell damage on the islet surface or mantle. Toxicity by hyperoxia tends to larger volumes compared with hypoxia. For example, the volume of hypoxic damage for an islet core 150 μm in diameter is 1.8×10^6 μm^3, whereas the volume of hyperoxic damage for an islet surface 25 μm in thickness is 6.0×10^6 μm^3, which is more than 3 times larger than the volume of hypoxic damage.

50% of medium O_2 concentration, respectively. These attempts are important to clarify the most appropriate media O_2 level for isolated islets, because the absolute pO_2 in the culture media varies depending on factors such as seeding density and media depth.[25–27] Consistent with the above in vitro results, in vivo studies also showed a beneficial effect of islet exposure to moderate hyperoxic condition. Better survival of rat islets in a subcutaneously implanted device was demonstrated by the reversal of diabetes in streptozotocin (STZ)-treated rats, where 40% O_2 was directly supplied via the device.[42]

These studies demonstrated that "air with 5% CO_2," the condition widely used for islet culture, is not sufficient for maintaining highly viable and functional islets, especially in large sizes. Optimal hyperoxic condition is beneficial. It is also important that the absolute pO_2 in the culture media is influenced by other factors, such as islet seeding density and media depth.[25–27]

OXYGEN TREATMENT OF ISLETS FOR TRANSPLANTATION

Human islets are isolated primarily for transplantation in T1D patients. During organ procurement, islets are exposed to hypoxic conditions starting from cross-clump, followed by pancreas cold preservation and islet isolation. In this section, we summarize efforts to alleviate islet hypoxia using O_2 treatment in the following 4 sections: (1) oxygenation of donor pancreas before islet isolation, (2) oxygenation of islets in the pretransplant culture stage, (3) solutions for oxygenation applicable in both pretransplantation and posttransplantation stages, and (4) posttransplant islet treatment by oxygenation.

Oxygenation Treatment of Donor Pancreas Before Islet Isolation

Many investigations aim to maintain donor pancreas quality during cold preservation to yield higher quality of islets in larger numbers. Immersion of a whole pancreas in oxygenated solution was utilized using several different solutions that can maintain higher O_2 levels for an extended period. The layer method using perfluorocarbon (PFC) is the most commonly used method. In this method the pancreas is placed at the interface between oxygenated PFC and cold preservation solution (University Wisconsin solution).[43,44] Oxygen is gradually released from PFC and penetrates into the pancreatic tissue. The original studies using canine pancreata achieved very impressive results. However, subsequent attempts by many islet transplantation centers to maintain human pancreas in the layer method failed to reproduce the effects and showed the limitation of this approach. This method depends on passive O_2 diffusion through the organ surface. Therefore, the thick human pancreata limits the beneficial effect of oxygenation.[45–47] Subsequently, perfluorohexyloctan, a semi-fluorinated liquid fluorocarbon, was compared with perfluorodecalin, a conventional PFC. With porcine pancreas, perfluorohexyloctan maintained higher intrapancreatic pO_2 (10.11 vs 1.64 mmHg with PFC) and improved islet viability and function, but it had no effect on islet yield.[48,49] Supplementation of intraductal L-glutamine to perfluorohexyloctan further improved preservation results and improved islet function.[50] Although islets isolated from the pancreas and stored in these solutions functioned better than the control islets, the results with porcine pancreata also revealed that simply immersing the organ limits the amount of oxygen available to the core of the organ.

Unlike passive O_2 diffusion from the organ surface, perfusion through blood vessels in the organ may be beneficial for uniform O_2 distribution. In fact, perfusion of a whole liver using a hypothermic machine has been extensively studied, and favorable oxygenation results were reported.[51–53] In a rat model, the liver was perfused for 18 hours using oxygenated solution equilibrated with 100% O_2. After transplantation, the liver functioned better and enzyme release was suppressed as compared with the controls preserved using the conventional method.[51] However, the perfusion of the pancreas is anatomically difficult because it has several feeding arteries. The involvement of exocrine tissue creates additional problems; in fact, a pancreas perfusion model has not been established.

A similar concept, oxygenation using a direct injection of gaseous O_2 into vessels (persufflation), was also attempted. This method was able to preserve multiple organs, including liver,

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kidney, and heart.[54–59] This approach was also applied to porcine pancreas to preserve the organ for 24 hours. Persufflation effectively reduced cell death as examined histologically, compared with the organ stored by the layer method.[45] A recent study using rats showed a better islet yield after the persufflation storage of the pancreas compared with static cold storage.[60] The persufflation of rat pancreas was performed from the portal vein and the organ was stored for 6 hours before islet isolation. However, the number of studies using perfusion or persufflation was limited for the pancreas preservation, and the best method to supply O_2 into the whole pancreas has not been established.

Oxygenation of Islets in Pretransplant Culture Stage

Isolated islets are usually placed in culture overnight or longer before being pooled and packaged for transplantation. The pO_2 of the culture influences islet survival and insufficient O_2 during culture can lead to islet cell necrosis. However, pO_2 in culture can be controlled to a desirable level for islets.

Culture in Hyperoxic Conditions

Central necrosis is often found in large islets that are cultured under normoxic air and 5% CO_2, the condition widely used for isolated islets. The major cause of the central necrosis is O_2 depletion or hypoxia, and the development of this hypoxic core can be easily prevented by culturing in higher levels of O_2 to induce hyperoxia.[18] However, because hyperoxic conditions can be toxic and lead to cell death on the islet surface, the O_2 concentration applied to islet culture must be carefully determined (Fig. 2).

As discussed in the previous section, increasing oxygen from 10% to 21% O_2 to 35% to 50% O_2 (270–350 mmHg pO_2) in culture media led to greater islet volume, viability, and function.[25] Thirty-five percent O_2 also maintained the best islet function evaluated by GSIS and higher islet volume than 50% O_2 culture, but with no statistical difference. In addition, other cell culture conditions require careful monitoring, including cell density and media depth, because the undesirable effect of high islet seeding/islet density may surpass the beneficial effect of O_2 supply.

Controlling Oxygen Effect by Islet Seeding Density and Culture Medium Depth

High islet seeding density induces O_2 depletion in culture media surrounding islets. When human islets are cultured at different densities (75, 300, and 600 IEQ/cm^2) under the same O_2 concentration, islets exhibit significantly different changes within 24 hours. These changes include decreased viability, decreased β-cell GSIS function, and increased proinflammatory chemokines released into culture media.[27] The expression of vascular endothelial growth factor, a hypoxic marker, increased in culture medium containing higher islet density. Similar results were also reported using rat islets cultured in densities ranging from 150 to 600 IEQ/cm^2, in which lower viability, decreased adenosine triphosphate level, and increased apoptosis were observed in higher density islet cultures.[26] This investigation also measured lowering the medium pO_2 to 80.5 mmHg in culture containing islets at 600 IEQ/cm^2, whereas pO_2 of 121.6 mmHg was maintained in culture containing islets at 150 IEQ/cm^2. These results are explained by the balance of O_2 supplied from the medium surface and O_2 utilized by islet cells, as demonstrated by in silico simulations.[15,25]

Medium depth is another factor limiting O_2 available to islets that settle at the bottom of the culture container. According to simulation data, there is a 30 mmHg decrease between the media surface and bottom of the well when 125 IEQ/cm^2 human islets are seeded in 5-mm-deep culture media.[25] Therefore, the islet seeding density and culture medium depth are limiting factors that determine islet viability and function by inducing an O_2 gradient in the medium.[61] To summarize, increased O_2 supply can be achieved by seeding islets at lower densities and by using a shallower depth of culture medium.

To reduce the O_2 gradient in culture medium, a vessel with O_2 permeable silicone membrane bottom was introduced as an effective system to culture islets at a high density.[62] This vessel supplies O_2 from the bottom of the culture vessel, providing higher islet oxygenation. Although this study did not measure actual pO_2, the quality of islets seeded at high density (4000 IEQ/cm^2) was well-maintained and comparable with that of islets seeded at low density (200 IEQ/cm^2) as evaluated by O_2 consumption rate/DNA. In another study, anodic aluminum oxide membranes with 14.6 nm pore size were fabricated; O_2 can permeate these membranes and immunoisolation is achieved by blocking passage of immunoglobulin.[63] Rat isolated islets cultured on the anodic aluminum oxide membrane showed high viability and function. Gentle stirring of islet culture or circulating culture medium may be the options to reduce the O_2 gradient; however, such processes may induce islet aggregation or damage, which should be avoided.

Solutions for Oxygenation Applicable in Both Pretransplantation and Posttransplantation Stages

To alleviate hypoxic islet injury, O_2 can be supplied by solutions that either generate or carry O_2 (direct oxygenation), or islets may be preconditioned to alleviate the damage. These approaches may be applicable in both pretransplantation and posttransplantation.

Oxygen Generating Materials

Oxygen generating materials are applicable as direct oxygenation procedures. Hydrogen peroxide is widely used for O_2 generating biomaterials ($2H_2O_2 \rightarrow O_2 + 2H_2O$). Oxygen-releasing microspheres were mixed in silicon elastomer (polydimethylsiloxane [PDMS], an O_2 permeable silicone material) to fabricate an O_2-releasing PDMS-H_2O_2 disc.[64] Improved insulin secretion was demonstrated by 3D culture using mice β-cells. Although this O_2 generating material is a potent approach, generation of O_2 was gradually lost over the course of the experiment. Thus, a longer-lasting O_2 generator is required for future use. In fact, several polymers have been introduced to control the O_2 release and to extend the release period.[65] A similar reaction was introduced with a poly-vinylpyrrolidone/H_2O_2 core and a poly(lactide-co-glycolide) shell.[66] Enzymes embedded on the surface of the microparticles not only provide sustained O_2 release but also reduce the risk of β-cell toxicity by H_2O_2. In addition, an angiogenic scaffold using fibrin-conjugated heparin/vascular endothelial growth factor collagen was applied to the microparticles leading to better survival in a mouse β-cell line and pancreatic rat islets. Enhanced angiogenesis and O_2 supply showed a synergic effect on the islet transplantation model using diabetic nude mice in omental pouch. Another chemical reaction has been utilized for isolated rat islets and other cells using calcium peroxide-based O_2-generating biomaterial.[67–69] A recent study showed that encapsulated solid calcium peroxide (PDMS-CaO_2 disc) generates O_2 for more than 6 weeks in the following reaction: $2CaO_2 + 4H_2O \rightarrow 2Ca(OH)_2 + 2H_2O_2 \rightarrow 2Ca(OH)_2 + 2H_2O + O_2$. Cocultured rat islets with the PDMS-CaO_2 disk in hypoxic condition maintained islet function measured by GSIS, suppressed cell death evaluated by lactate dehydrogenase release, and lowered caspase activity after 8- to 48-hour culture. Rat isolated islets (1800 IEQ) were cocultured in high seeding density (1330 IEQ/cm^2) with this biomaterial under hypoxic condition (0.01 mM O_2) for 24 hours, followed by syngeneic transplantation on the omentum. Transplant using 24-hour–cultured

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islets under normoxia using the identical seeding density was compared as a control, and diabetes was reversed only in the treatment group. In the study, the disk was used as a preconditioning material (pretransplant culture); therefore, additional development of the cotransplantable disk is warranted for long-term O_2 supply to improve the hypoxic microenvironment of the transplant site. A chemical reaction using sodium percarbonate ($2Na_2CO_3 \cdot 3H_2O_2$) was also tested as another material to generate O_2. It was initially used in a poly(lactide-co-glycolide scaffold to prevent cell death in the rat muscle injury model.[70,71] However, it has not yet been tested for oxygenation of transplanted islets.

Photosynthesis of thermostable microalga (*Chlorella sorokiniana*) was introduced as a means to supply O_2 to cultured islets coencapsulating in alginate gel.[72] Based on their calculation, 13,000 alga cells under saturating light produce the amount of O_2 consumed by a single islet, and islets coencapsulated with microalga showed better GSIS function. A photosynthesis reaction was also introduced for the transplantable islet chamber using *Synechococcus lividus*, a thermophilic cyanobacterium.[73] This chamber, consisting of a small light source and islet compartments (3000–4000 IEQ of syngeneic islets), was implanted subcutaneously into STZ-induced diabetic rats and was able to reverse diabetes in this model.

Oxygen Carriers

Direct oxygenation methods include O_2 carriers. Oxygen carriers, such as perfluorocarbons and Hb, have been tested in islet culture for their capacity to provide additional O_2.[29,74–76] Recently, a novel attempt of perfluorodecalin (PFD)–based oxygenation was introduced for the culture of rat islets.[77] Islets were cultured in autologous plasma-based matrix, or the same matrix supplemented with PFD, to test increased O_2 supply. After 24 hours, islets in the matrix with PFD showed higher matrix-cell contact, viability, and function and decreased translocation of HIF-1α in the nucleus compared with controls in the matrix alone. These islets were syngeneically transplanted into the liver, after the PFD-plasma construct was dissolved by plasmin. Although PFD-plasma–treated islets accelerated unfavorable instant blood mediated inflammatory reaction in the model, this approach may be effective to transplant islets in extrahepatic sites where islets are not exposed to the blood stream.

Oxygenation of cell culture can also be achieved using Hb-based molecules that released O_2 on demand. HEMOXCell, a commercially available technology (Hemarina, Boston, Mass) is a marine macro-Hb capable of carrying 156 O_2 molecules in a ready to use solution. A recent study revealed both PFD and Hb solutions effectively alleviate hypoxia. However, HEMOXCell showed better insulin secretion in isolated rat islets cultured in vitro under 2% O_2 than PFD.[78] Polymerized Hb is a biodegradable O_2 carrier that was introduced as an erythrocyte substitute; however, several adverse effects have been reported including central nerve and cardiovascular systems when systemically administered.[79] On the other hand, hypoxia-mediated β-cell apoptosis was alleviated when poly-Hb was coplaced locally with islets in mice.[80] When transplanted in abdominal muscle, the loss of syngeneic islets was decreased by addition of poly-Hb in a dose-dependent manner as evaluated by pimonidazole (hypoxia marker) using immunohistochemistry. The islet graft function was also improved as shown by intravenous glucose tolerance test. However, a concentrated poly-Hb was found to induce β-cell apoptosis as early as a day after the transplantation, and thus the appropriate dose was optimized in this study.

Preconditioning by Hypoxia Exposure

To alleviate islet loss caused by hypoxic injury, various types of preconditioning methods have been proposed. Exposure of β-cells/islets to hypoxia was found effective and widely used for islet preconditioning. To elucidate the mechanism of action, INS-1 cells were exposed to hypoxia (0.3%–0.5% O_2) for 18 hours followed by 20 to 22 hours of reoxygenation.[81] These pretreated INS-1 cells increased substrate-stimulated respiratory capacity and coupling efficiency. In islets, these effects were accompanied by up-regulation of mitochondrial complex protein levels and increased respiratory capacity after reoxygenation. Because the hypoxic exposure itself is harmful, a method to reduce hypoxia toxicity was developed for islet preconditioning by designing a diffusion-based microfluidic device, allowing a short-period intermittent exposure of islets to hypoxia.[82] Using this device, mouse islets were preconditioned for 30 minutes using intermittent 1-minute exposures to 5% and 21% O_2 before placement under hypoxia. The effect of this intermittent hypoxia preconditioning showed better insulin release function in response to high glucose than untreated islets, demonstrating the effectiveness of preconditioning to induce hypoxia resistance with reduced cell toxicity.

As described previously, hypoxia causes calcium influx into islet cells, leading to cell damage.[35] Therefore, pharmacological means to alleviate a negative impact of hypoxia have also been investigated to block this calcium influx. A potassium channel opener/activator (diazoxide) competes with calcium and suppresses calcium influx. Rat islets exposed in hypoxia (0.8% O_2 for 5.5 hours) decreased proinsulin biosynthesis by 35%; however, preconditioning counteracted this decrease by 91%. Syngeneic transplantation of diazoxide-preconditioned islets demonstrated better control of blood glucose compared with nonpreconditioned islets in STZ-treated rats. Similar result was obtained using preconditioned human islets transplanted to STZ-treated nude mice. A calcium channel blocker, nifedipine, partially reproduces the effects of diazoxide. These Food and Drug Administration–approved and clinically-established agents appears to have therapeutic potential for clinical islet transplantation.[35,83]

Cytoprotective Agents/Chemicals to Protect Islet Damage From Hypoxia

Cytoprotective molecules are introduced to alleviate the hypoxia-induced islet damages. The organic compound, curcumin, was tested for its ability to protect β-cells from hypoxic injury.[84] As tested in INS-1 cells, curcumin administered through peptide micelles prevented β-cell apoptosis by suppressing ROS elevation. Metformin is known as a potent antihyperglycemic agent and improved insulin sensitivity in T1D patients.[85,86] γ amino butyric acid (GABA) upregulated PI3K/Akt-dependent growth and survival pathways via membrane depolarization of islet β-cells,[87] and improved the mouse and human islets survival and supports function.[88] Dietary intake of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) was beneficial to reduce the development of autoimmunity in children at increased genetic risk for T1D.[89] In recent study, combinations of these molecules (EPA + DHA + GABA and EPA + DHA + Metformin) reduced ROS production induced by hypoxia (1% O_2 for 48 hours) in 3D islet like clusters derived from human umbilical cord of mesenchymal stem cells.[90] Another organic chemical, puerarin (a major isoflavonoid), alleviated β-cell apoptosis and malfunction by hypoxic injury of β-cells in obese mice induced by cobalt chloride induced via PI3K/Akt pathway activation.[91] Orally administered puerarin also activated the glucagon-like peptide1receptor pathway and subsequent Akt activation, leading to improved mouse β-cell survival.[92] In another study, the administration of puerarin reduced hypoxia-mediated injury in a rat model for cerebral artery occlusion and suppressed HIF-1a, the inducible nitric oxide synthase and tumor necrosis factor-α in the ischemic region.[93]

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Therefore, puerarin may also be useful to reduce HIF-1a–associated hypoxic injury of islets.

Posttransplantation Islet Treatment by Oxygenation

There is no doubt that transplanted islets are exposed to hypoxia; most of the graft sites have a pO_2 too low to support islet survival. Even the liver, the conventional transplant site, has a pO_2 of only 40 to 50 mmHg.[94] Subcutaneously placed islets also suffer from hypoxia around 45 mmHg.[95] If islets do survive, the islet capillary networks recover and establish a connection to the recipient vascular system. However, this requires 10 to 30 days, depending on the animal species and transplantation site.[96–98] Providing appropriate O_2 to transplanted islets is far more complicated than solving the hypoxia problem in vitro because (1) precise measurement and control of local pO_2 is difficult, (2) islet survival depends on O_2 diffusion through the surrounding tissue as well as O_2 transported by Hb, and (3) islet survival is also affected by other factors, such as inflammatory reactions. For these reasons, there are few methods that have successfully improved in vivo islet survival after transplantation. However, unique in vivo–specific treatment methods have been developed and tested in animal models. These include localized oxygenation used in bioartificial pancreas and systemic oxygenation (O_2 inhalation).

Localized Oxygenation at the Graft Site

A chamber was developed as a bioartificial pancreas to implant islets subcutaneously. Islets seeded in the chamber were provided O_2 and nutrients and protected from immune reaction.[42,99,100] The system consists of a mechanical shell holding the islets embedded in hydrogel and a gas chamber that supplies O_2. The shell was covered by a Teflon membrane (0.2 μm pore size) to achieve immunoisolation. Oxygen was provided through an O_2 permeable membrane from a gas chamber that was filled with a desired concentration of O_2 using ambient air (21% O_2) or increased oxygen (40% O_2) to support subcutaneously placed islets. Using this device, 2200 to 2600 of syngeneic rat islets reversed diabetes with 40% O_2 supply, but not with 21% O_2, indicating the importance of the O_2 concentration for islet survival. However, diabetes reoccurred when the 40% O_2 supply was terminated, which indicates that no O_2 was supplied via Hb and that blood vessels did not reconnect to the graft. In this context, this device is considered as a transplantable islet culture system in which islets relied on the externally supplied O_2. This device also demonstrated its ability to immunoisolate islets by survival of allogeneic islets in rats and minipigs.[99,100] In terms of the O_2 concentration, higher levels of O_2 showed better results, especially when islets were densely seeded. An islet seeding density and O_2 concentration must be taken into consideration for fabricating such devices.[61] This is also a universal issue in extrahepatic islet transplantation in which islets are transplanted at high density. Recently, we developed a new O_2 transporter and tested it on syngeneic islets transplanted into rats subcutaneously.[101] This device was made of O_2 permeable PDMS consisting of 3 parts: an O_2 receiver, an O_2 diffuser, and a cannula connecting both. The receiver is placed in the hypoxic space (eg, subcutaneous site), and the O_2 diffuser is placed in a higher O_2 environment than the receiver (eg, ambient air) (Fig. 3A, B). Oxygen is transported from the receiver to the diffuser by the O_2 gradient, without electricity or chemical reaction. Simulation revealed that the pO_2 in the islet layer in the subcutaneous (SC) transplant site increased to 55 mmHg on the O_2 receiver, which contained 5000 IEQ/cm^2 islets. In contrast, the pO_2 of the islet transplant site without O_2 transporter was 3.7 mmHg. Islets placed on the O_2 diffuser were transplanted subcutaneously (Fig. 3C), and in vivo viability of islets was more than 3-fold higher than the controls

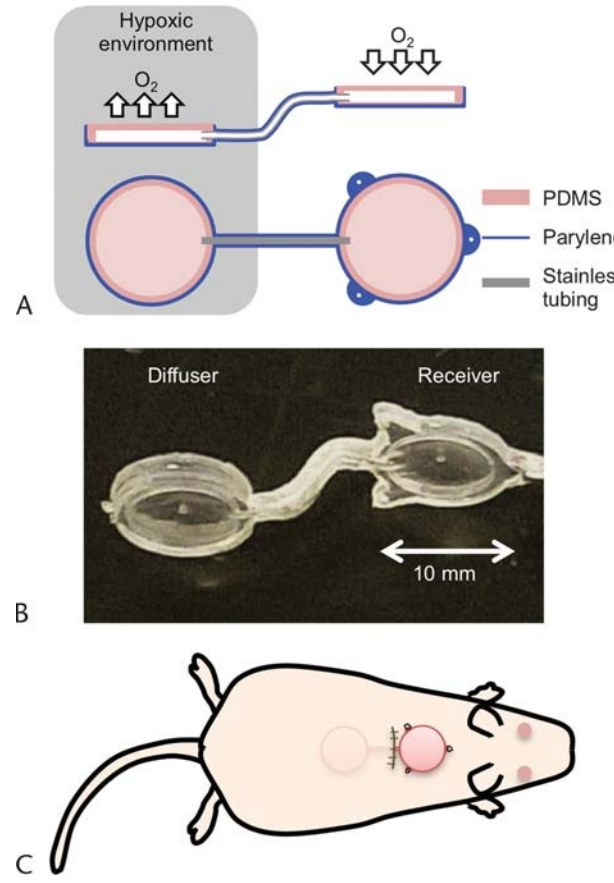

FIGURE 3. Oxygen transporter device for localized oxygenation at the graft site. A, Schematic of the O_2 transporter. The transporter consists of 3 parts: receiver (right), diffuser (left), and a connecting cannula (middle). The device is made of O_2 permeable PDMS and coated by O_2 impermeable Parylene except for the upper surfaces of the receiver and diffuser. Oxygen is taken up through the receiver and released through diffuser. An islet graft can be placed on the diffuser. Oxygen is transported by diffusion; therefore, this system does not require a power source and is not driven by a chemical reaction. B, Image of the O_2 transporter. C, Schematic of a rat carrying the O_2 transporter on its back. The receiver is exposed to ambient air, and the diffuser with a graft is subcutaneously implanted.

placed subcutaneously without O_2 transporter as tested 7 days and 14 days after transplantation. This simple device and approach shows great promise to improve islet survival after subcutaneous transplantation with fewer complications. In addition, the device can be fabricated at low cost.

Systemic Oxygenation of Islet Recipients

Inhalation of high O_2 can achieve systemic oxygenation and increase tissue pO_2 throughout the body. Oxygen inhalation treatment is commonly applied for the recovery process from general anesthesia and postsurgery, as well as for patients with respiratory disorders. The O_2 used for treatment ranges from 21% to 100% under normobaric pressure (1 atm). Hyperbaric O_2 therapy (HBO, 2 ~ 3 atm) is also used for treatment of several diseases such as decompression sickness caused by diving and refractory wounds.[102–104] Because many publications and reviews are available on HBO, this review only discusses the recent advancement of normobaric O_2 therapy associated with islet transplantation.

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The effect of O_2 inhalation on tissue O_2 was studied in postoperative patients.[105–107] pO_2 in the wound after mastectomy was measured below 40 mmHg in ambient air (21% O_2), but increased to 60 mmHg by 50% O_2 inhalation. In the abdominal wound, pO_2 also increased to 60 to 100 mmHg by 70% to 80% O_2 inhalation. These results clearly indicate that tissue pO_2 responds to O_2 inhalation. Partial O_2 tension measured in these studies was in the postoperative wound where the vascularity was deranged; therefore, the response of subcutaneous tissue may be greater and more sensitive to O_2 inhalation. We studied the effect of systemic oxygenation on the SC islet transplantation using syngeneic islet transplantation in rats.[95] Inhalation of 50% O_2 increased SC pO_2 to 140 mmHg from 45 mmHg measured in control rats inhaled 21% O_2. A continuous 50% O_2 treatment for 3 days reversed diabetes with 600 islets/rat (3500 IEQ/kg), whereas controls without O_2 treatment remained diabetic. Mimicking the subcutaneous O_2 environment, rat islets were cultured in vitro in medium pO_2 (140 or 45 mmHg), which was equivalent to the SC O_2 environment under 50% or 21% O_2 inhalation, respectively. In vitro results demonstrated that 140 mmHg O_2 prevented the development of central necrosis of the islets, maintained higher cell viability, and better β-cell function as measured by GSIS compared with those cultured in 45 mmHg. Therefore, posttransplant O_2 treatment after SC transplantation supports islet survival and functionality, but the length of O_2 inhalation treatment in rats was limited to 3 days to avoid toxicity to lung and brain.

Re-establishment of intraislet capillary networks is essential to prevent chronic hypoxia and to sustain graft function. Previous studies have shown the effectiveness of prevascularization of the graft bed for SC islet graft survival.[108–111] Importantly, with the help of systemic oxygenation and prevascularized graft bed, a small islet number (3500 IEQ/kg) was able to reverse diabetes in similar models of rat syngeneic SC transplants.[108,110,112] In addition, this number is comparable to the islet number of rat intraportal islet transplant (2500–3500 IEQ/kg),[113–116] which indicates the SC site can be an alternative site. This site can be accessed using a minimally invasive procedure, and the site is easy to monitor while modulating O_2 concentration.

Oxygen inhalation was also applied to the conventional intraportal islet transplant in rats.[116] Rats were housed in a 100% O_2 environment for 2 days after syngeneic islet transplantation. Although the actual pO_2 in the treated animals was not measured in this study, this treatment decreased the islet number required for diabetes reversal from 1000 islets (21% O_2 inhalation) to 700 islets (100% O_2 inhalation) per rat. Another study showed that the vascularity of human islets intraportally transplanted in nude mice was only 30% compared with that of the native islets, and low vascularity was correlated with the hypoxic area examined by the histology.[117] These studies demonstrate the poor oxygenation and vascular recovery in the conventional liver site and beneficial effects of oxygenation on intraportal islet transplantation outcome.

Collectively, systemic oxygenation appears a potent option as a posttransplant treatment. It is an established, easy and cost-effective method to treat patients. Normobaric O_2 is less toxic compared with the HBO; however, treatment period and O_2 concentration need to be optimized for the best therapeutic outcome.

SUMMARY

The proper supply of O_2 is the key factor for improving islet survival and physiology in any sites used for transplantation. Recent studies showed that O_2 provides favorable effects in all steps of the transplantation process, from pancreas procurement to posttransplantation. Biomaterial to supply O_2 is an emerging field, and in silico simulation has contributed to our understanding of islet physiology. In addition, simple and already available methods to alleviate hypoxic injury that often accompanies transplantation, such as adjustment of islet seeding density, culturing isolated islets under hyperoxic conditions, and posttransplant O_2 inhalation therapy, have proven effective in preserving islet function. These methods can be easily introduced into clinical islet transplantation. There are many choices for supplying O_2 as surveyed in this review. However, further determination of the optimal O_2 supply required at different stages of islet physiology and islet location is required to develop better treatments using specific and targeted O_2 to increase the therapeutic success of islet transplantation.

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