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Applying Immunomodulation to Promote Longevity of Immunoisolated Pancreatic Islet Grafts

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Islet transplantation is a promising therapy for insulin-dependent diabetes, but large-scale application is hampered by the lack of a consistent source of insulin-producing cells and need for lifelong administration of immunosuppressive drugs, which are associated with severe side effects. To avoid chronic immunosuppression, islet grafts can be enveloped in immunoisolating polymeric membranes. These immunoisolating polymeric membranes protect islet grafts from cell-mediated rejection while allowing diffusion of oxygen, nutrients, and insulin. Although clinical trials have shown the safety and feasibility of encapsulated islets to control glucose homeostasis, the strategy does up till now not support long-term graft survival. This partly can be explained by a significant loss of insulin-producing cells in the immediate period after implantation. The loss can be prevented by combining immunoisolation with immunomodulation, such as combined administration of immunomodulating cytokines or coencapsulation of immunomodulating cell types such as regulatory T cells, mesenchymal stem cells, or Sertoli cells. Also, administration of specific antibodies or apoptotic donor leucocytes is considered to create a tolerant microenvironment around immunoisolated grafts. In this review, we describe the outcomes and limitations of these approaches, as well as the recent progress in immunoisolating devices.

Keywords: diabetes, pancreatic islets, islet transplantation, immunoisolation, encapsulation, immunomodulation

Impact Statement

Immunoisolation by enveloping islets in semipermeable membranes allows for successful transplantation of islet grafts in the absence of chronic immunosuppression, but the duration of graft survival is still not permanent. The reasons for long-term final graft failure is not fully understood, but combining immunoisolation with immunomodulation of tissues or host immune system has been proposed to enhance the longevity of grafts. This article reviews the recent progress and challenges of immunoisolation, as well as the benefits and feasibility of combining encapsulation approaches with immunomodulation to promote longevity of encapsulated grafts.

Introduction

Type one diabetes (T1D) is a metabolic disorder resulting from autoimmune destruction of β cells in pancreatic islets.[1] Injection of insulin is the current standard therapy for T1D, but it remains a challenge to control the patients' blood glucose levels as strictly as in healthy individuals. Long-term insufficient glycemic control causes chronic complications.[2] Hypoglycemia is also a major complication of the current insulin therapy and causes 4–10% of all the deaths in T1D patients.[3–6] Hence, establishing a therapy to accomplish tight glycemic control is an urgent need.

Transplantation of pancreatic islets provides a minute-to-minute regulation of glucose levels, which might prevent many of the side effects of insulin therapy.[7] Since the introduction of the Edmonton Protocol using steroid-free immunosuppression in 2000, the rate of successful islet engraftment significantly increased.[8] However, there are still

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major obstacles to overcome such as low survival rates of islet grafts,[9] low availability of high-quality and well-characterized donor islets,[10] and the need for lifelong administration of immunosuppressive drugs.[11] Especially, the lifelong immunosuppression leads to severe complications, such as pathogenic infections or malignancies.[12,13]

A conceivable approach to overcome the need for chronic immunosuppression is immunoisolation (Fig. 1A) by encapsulating islets within biomaterials that form a semipermeable membrane. The membranes do not allow entry of immunoglobulins and effector cells but do allow free passage of nutrients, glucose, and insulin. Although success with these membranes has been shown in animal models and humans,[14] large-scale application of encapsulated islet transplants is limited due to too short graft survival times that do not exceed months,[15] and it is still not entirely understood why encapsulated islet transplants fail.

Immune responses activated by humoral factors, such as damage-associated molecular patterns (DAMPs) from necrotic or necroptotic cells in the capsules and cytokines released during immune responses initiated by the mandatory implantation surgery and/or early foreign body responses (FBRs), are suggested to contribute to significant loss of islet cells in the immediate period after implantation and may shorten longevity of the graft.[16–19] To make encapsulated islet grafts a feasible option for treatment of T1D, we need to prolong graft survival. Therefore, immunoisolation is sometimes combined with immunomodulation (Fig. 1B) to attenuate host immune responses against islet grafts in the immediate period after transplantation and by that reducing loss of islets.

In this review, we first discuss and review current improvements in encapsulation approaches to promote cell survival. After that, we review combined strategies of immunoisolation and immunomodulation and discuss the outcomes and limitations.

Immunoisolation

Current immunoisolation technologies can be divided in two separate approaches, macroencapsulation and microencapsulation (Fig. 2), which are discussed in the sections below.

Macroencapsulation

Macroencapsulation devices contain multiple islets inside an immunoisolating membrane and can be further subdivided in intravascular and extravascular devices.[20] They can be formed by various types of polymers, for example, polytetrafluoroethylene (PTFE), alginate, polysulfone, polyacrylonitrile, and polyvinylchloride.[21–24]

Intravascular macroencapsulation devices exist from a semipermeable membrane that is connected to the host's blood vessels.[25] In this way, islets are in direct contact with the recipients' blood flow, which enables fast exchange of molecules.[26] One of the hurdles for clinical application of intravascular devices is the formation of blood clots in the lumen of the device due to adsorption of proteins on the luminal polymers.[27,28] Song *et al*. demonstrate that this can be reduced with the inclusion of a nanopore membrane,[29] but intravascular devices still have a high risk for infections and also require invasive and complex surgery.[30,31]

Extravascular macrocapsules also have multiple islets in their lumen but are not in direct contact with the bloodstream of recipients. The macrocapsules, which are available in different geometries, for example, fibers, sheets, and diffusion chambers,[23,32,33] are implanted with minimal invasive surgery under the skin or into the abdominal cavity and can be removed if necessary. An issue with macrocapsules is the rather low and slow diffusion of nutrients and oxygen to encapsulated tissue compared with microcapsules, because of the unfavorable surface-to-volume ratio of macrocapsules.[34] This leads to hypoxia, gradual ischemia of the encapsulated cells, and finally, graft failure. To ensure optimal supply of oxygen, it is suggested that the diffusion distance, the distance between blood vessels and the islet cells in the capsules, should be <100 μm.[35]

Several research groups have developed strategies to solve the limited oxygen diffusion by promoting neovascularization. The strategies are to provide oxygen such as applied in the TheraCyte™ device from TheraCyte, Inc.,[21,36,37] by supplementing exogenous oxygen to the device as in the βAir device,[22,38,39] or by implanting the islets into a prevascularized site.[40,41]

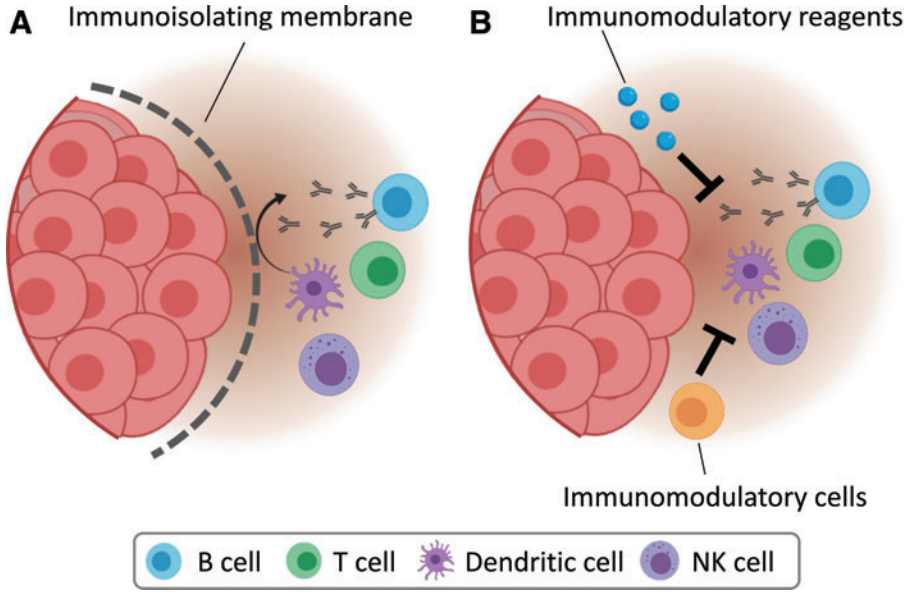

FIG. 1. Schematic presentation of two approaches to protect islet grafts from the host immune system. **(A)** Immunoisolation prevents rejection by blocking immune cells using a semipermeable immunoisolating membrane. **(B)** Immunomodulatory reagents or cells modulate local immunity and reduce rejection of islet cells. NK cell, natural killer cell. Color images are available online.

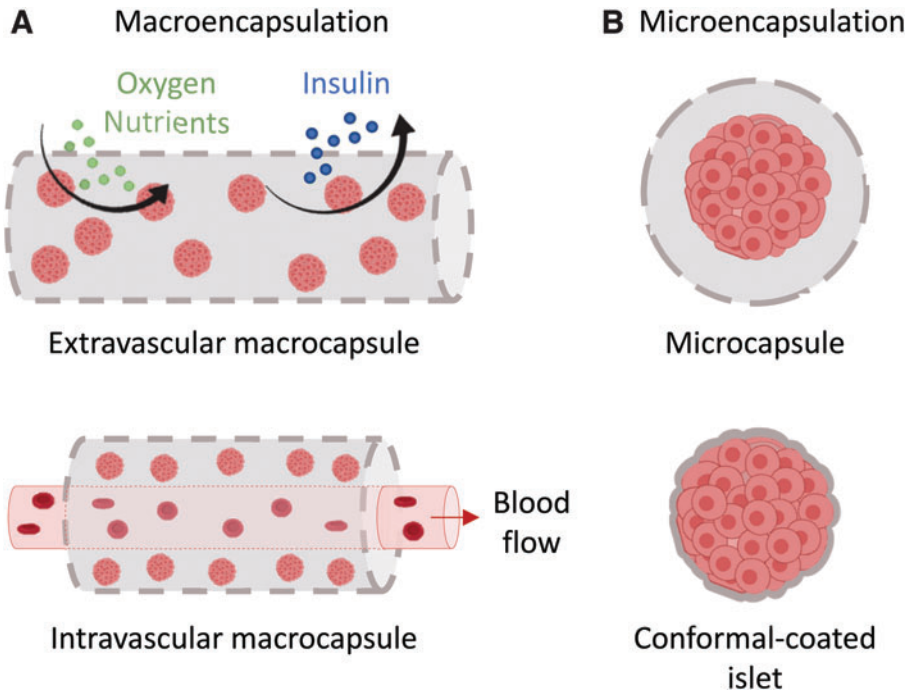

FIG. 2. Categories of immunoisolation technologies. Schematic images of representative immunoisolating capsules of **(A)** macroencapsulation: an extravascular or an intravascular macrocapsule and **(B)** microencapsulation: microcapsule and conformal-coated islet. Color images are available online.

For example, a prevascularized site was constructed by basic fibroblast growth factor (bFGF)-impregnated microspheres under the skin of diabetic mice.[40] Rat islets were encapsulated in an agarose and polystyrene sulfonic acid-based macrocapsule and implanted into the prevascularized site. The macroencapsulated islet-xenograft implanted into the site demonstrated a significantly longer graft survival period than the untreated group. Also, a bFGF-loaded bioabsorbable device[42] and a poly (D,L-lactide-co-ε-caprolactone)-based scaffold[43] were proposed to accelerate revascularization of islets and contribute to better oxygenation of islets in the device. As islets are metabolically highly active, access to vasculature is essential for graft survival and function.[44,45] Several methods to accelerate access to vasculature have been suggested such as cotransplantation, coaggregation, and coating of islets with vascularization supporting cells. These might all be helpful to accelerate nutrition and oxygen diffusion into encapsulated islet grafts.[46]

Proof for clinical safety of oxygen-enriched macroencapsulated islet graft

The clinical safety of macroencapsulated islets in a device to enhance oxygen supply has been confirmed in 2010s.[47] In 2014, a Phase I/II clinical trial (NCT02239354) was launched to test the safety of a PEC-Encap (VC-01) device in humans with human stem cell-derived β cells in the absence of systemic immunosuppression. Although this was a safety study, it was shown that the device required improvements as many of the encapsulated cells died a few months after transplantation because of hypoxia caused by overgrowth on the outer membrane of the device.[48,49] In 2017, ViaCyte launched a new clinical trial (NCT03162926, NCT03163511) with a new macroencapsulation device. As the device is porous and allows blood vessels to directly enter the grafts, systemic immunosuppression is required. However, all data show until now the safety of the device, which was the primary goal of the study.[47]

Another clinical approach to enhance oxygen supply was applied in the so-called βAir device.[39] The βAir device has three compartments. The central compartment is separated from the two-sided compartments with silicone rubber membranes. The two-sided compartments surrounding the center are composed of porous PTFE membranes impregnated with alginate and contain multiple human islets. The central compartment is connected to an oxygen access port and an oxygen gas mixture is used to fill the central compartment on a daily basis.

In 2012, a clinical trial was performed and human islets encapsulated into a βAir device were transplanted in the preperitoneal site in a T1D patient. The islet graft was viable for 10 months and the device was covered with only a thin layer of cellular overgrowth, which was well vascularized without any signs of inflammation.[22]

In 2014, another Phase I study was performed to assess the safety of the βAir device with human islets. Four T1D patients received one or two βAir devices with human islets in the subcutaneous site and were monitored for 3–6 months.[38] Although also in this study the safety of the βAir device was demonstrated and islet grafts survived in the devices, only transient levels of circulating C-peptide were observed, while no impact was observed on insulin requirements and hemoglobin a1c (HbA1c) levels. The HbA1c levels are used to determine improvements in average plasma glucose concentrations during periods of 1–2 months. A factor contributing to this lower glycemic impact might have been the cellular overgrowth of the device, but also differences in islet quality between the first and second trials and the number of islets in the device, as well as the use of different implantation sites in the first and second clinical trials.

Microencapsulation

Microcapsules just contain one or a small number of islets, while in macrocapsules, larger numbers of islets are encapsulated. Microcapsules can be formed by various types of natural polymers, for example, alginate, agarose, and cellulose[50–52] or synthetic polymers, for example, polyethylene glycol (PEG) and polyvinyl alcohol.[53,54] The traditional way of encapsulating islets is by suspending the islets in a solution of one of the aforementioned polymers and to transfer them into droplets. These droplets are collected in a polymerization solution to form solid capsules.[55]

Advantages of microencapsulation over macroencapsulation are the larger surface-to-volume ratio, lesser invasive surgery, higher mechanical stability,[56] a safer encapsulation procedure with nontoxic molecules and agents,[56] and the relatively thin layer surrounding the islet graft that maximizes diffusion of oxygen and nutrition. There is an additional advantage of microcapsules, which is the fact that if one of the microcapsules fails, the others still function.[57,58]

Other microencapsulation strategies

Nowadays, there are microencapsulation technologies that are not based on droplet formation.[53,59] Intensive research is being done on, for example, conformal coating and layer-by-layer approaches. Both technologies hold the advantage of a further decrease of the diffusion distance, to improve oxygen and nutrient transport.

PEG is commonly used for the conformal coating of islets with photopolymerization[53] or with microfluidic systems.[59] Neocrin, Inc. successfully transplanted conformal-coated porcine islets, which were coated with PEG crosslinked with ultraviolet/visible light, into diabetic rodents.[60] The strategy, however, was not successful in larger animal models due to the incomplete coating of islets.[61] To protect the islet graft from rejection in larger animal models, Novocell improved the crosslinking.[62] Allogeneic subcutaneous transplantation of conformal-coated islets using this approach resulted in normoglycemia in a preclinical trial with nonhuman primates (NHPs).

As single-layer coating was associated with incomplete encapsulation, the layer-by-layer approach was developed.[63] Layer-by-layer coating of NHP islets with three layers of PEG formed a uniform nanoshield on the surface of the islets without impairing islet viability. Immunocompetent rodents receiving the coated islets resulted in 100% survival rates for 150 days after transplantation, but systemic immunosuppression was required.[64]

Proof for clinical safety of microencapsulated islet grafts

To investigate if microencapsulated islet grafts have a possibility to improve glycemic control of T1D patients, clinical trials using microencapsulated islets were started in the 1990s. It should be emphasized that most of the trials up to now were aiming on demonstrating safety rather than efficacy, with the exception of one study.

In 1994, human islets encapsulated within alginate-polylysine-alginate were transplanted into the abdominal cavity of a T1D patient.[65] This transplantation resulted in insulin independency without any complications for 9 months, demonstrating proof of principle.[65] More studies are urgently needed to determine the efficacy of immunoisolated islet grafts in restoring long-term normoglycemia in humans. Also, a Phase I/II clinical trial with two T1D patients was performed in 2005, in which PEG conformal-coated human islets were transplanted under the human skin without systemic immunosuppression. Although also graft-unrelated factors might be responsible for the beneficial effects, the patients demonstrated significant reductions in their insulin dose requirements for 4 and 6 months, but insulin independence was not achieved.[62]

Another group performed transplantations of encapsulated porcine islets in alginate-polylysine-alginate into a T1D patient.[66] At 12 weeks after transplantation, the insulin requirement decreased by 30% but returned to pretransplant requirements by week 49. In 2007, encapsulated neonatal porcine islets in alginate-based microcapsules were transplanted into eight T1D patients, of whom six patients experienced a reduction in insulin requirement.[67] Following that, Phase I/IIa and IIb clinical trials were started. In 2016, two transplantations of neonatal porcine islets in alginate-based microcapsules were performed in four T1D patients in two different doses.[68] All patients demonstrated improvements of HbA1c levels and reduced hypoglycemic unawareness especially in the high islet dose group. This might be caused by a higher glucagon release of the high-dose grafts, which improves glucose counterregulation.[69–71] The failure of some encapsulated islets might be related to an overgrowth by fibrotic and inflammatory cells on the surface.

Although all the microencapsulation clinical trials with alginate have shown their safety and improvement of glycemic control, long-term insulin independence has not been shown up to now, but this is due to the fact that none of the abovementioned studies was designed to demonstrate efficacy.[68,72] Now that the field has progressed to a point that safety has been demonstrated, clinical trials should start with optimal islet doses to show efficacy of microencapsulated islet grafts.

Toward prolonged longevity of islet grafts encapsulated within an immunoisolating membrane

All the aforementioned immunoisolation studies illustrate a common challenge, which is extension of graft longevity. Graft survival was not permanent and limited to several months up to a maximum of 1 year post-transplant.[15] The reason behind the failure of encapsulated grafts is still not fully known, but the FBR against polymers used for encapsulation is considered one of the reasons why encapsulated grafts fail. FBR causes pericapsular fibrotic overgrowth (PFO), which leads to hypoxia, lack of nutrient supply, and ultimately, graft failure.[55] To improve encapsulated graft survival, it will be essential to minimize the immunogenicity of the polymer(s) used for encapsulation.

Several studies have been optimizing polymer purity,[73] capsule geometry,[26,74] and chemical composition of the encapsulation polymers[75] and have applied chemical modifications of the capsule surface to improve biocompatibility.[74,76–78] Especially, the biocompatibility of alginate microcapsules strongly depends on the chemical composition of alginate.[26,75] Alginate is made from units of guluronic (G) and mannuronic (M) acid, and alginate microcapsules composed of high-G alginate are more stable

than high-M alginate and intermediate-G.[56,75] However, microcapsules from intermediate-G are one of the few alginate types that do not cause PFO.[75]

Apart from the property of alginate, another reason for graft failure might be the stimulation of immune responses by encapsulated islets (Fig. 3A). DAMPs, for example, DNA-fragments and uric acid, stimulate a cascade of proinflammatory signaling pathways, inducing activation of nuclear factor kappa B.[79,80] This leads to production of proinflammatory cytokines and chemokines and provokes activation of both the innate and adaptive immune systems resulting in islet cell death.[81–83] DAMPs from encapsulated islet grafts stimulate immune cells, including T cells, and attract especially macrophages, granulocytes, and fibroblasts to attach to the capsular surface resulting in PFO (Fig. 3A).[73] This leads to hypoxia and finally cell death.

In addition, indirect antigen presentation might occur and contribute to responses in case of xenografts (Fig. 3B). Immunoisolating membranes can inhibit cell–cell-mediated rejection with direct antigen recognition by host T cells. However, if xeno-epitopes pass through the membrane and are presented by host antigen-presenting cells, the recruitment and activation of graft-specific T cells such as $CD4^+$ and $CD8^+$ T cells may still occur.[84,85] Especially, $CD4^+$ T cells contribute to graft rejection of encapsulated neonatal porcine islets by the mouse host immune system.[86] Although it is difficult to quantify the actual concentrations of cytokines in capsules or in the transplantation site, the stimulated immune cells might activate inflammatory reactions and produce proinflammatory cytokines and chemotactic molecules, resulting in the cell death of encapsulated grafts without overgrowth on the surface of capsules.[17,18]

Several studies have shown that the cytokine release induced by the aforementioned processes may have a drawback on islet function.[87–89] In a recent study, we demonstrated that inclusion of a polymer on the surface of microcapsules that reduced toll-like receptor (TLR)2-1-induced immune responses can attenuate both *in vitro* and *in vivo* release of cytokines and enhance islet function and survival of the grafts.[90] Although to prevent many of the discussed immune responses is difficult only by encapsulation approaches and it is still unknown to what extent these responses contribute to reduced longevity, it is needed to find means to prevent the effects of these responses on islet survival. This has been a motivation for the research community to combine immunoisolation approaches with immunomodulating approaches to promote longevity of encapsulated islet grafts.

The Combination of Immunoisolation and Immunomodulation

Immunmodulation comprises a family of technologies aiming at regulating the immune response by the host against grafts to avoid long-term systemic immunosuppression. In the 1990s, researchers started to combine immunomodulating and immunoisolating approaches.[91–93] For example, they combined encapsulated islets and treatment of recipients with the anti-CD4 monoclonal antibody (mAb)[91] or immunosuppressive drugs.[92] This resulted in longer graft survival times than with any of the approaches alone. Another group transplanted alginate-encapsulated adult porcine islets into nonobese diabetic (NOD) mice with CTLA4-Ig and anti-CD154 mAb treatment. This strongly suppressed the PFO on the surface of the capsules and prolonged graft survival.[93] These studies suggest that the combination of immunoisolation and immunomodulation may improve long-term encapsulated graft survival. During recent years, new approaches for immunomodulation have been emerging. These involve inclusion of immunomodulating cells and administration of novel molecules. In this section, we review several

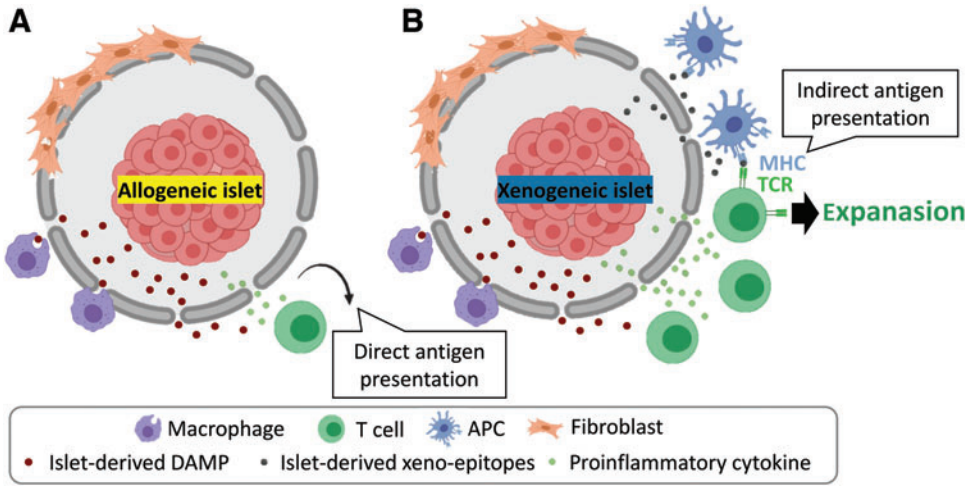

FIG. 3. Immune responses against encapsulated islets in immunoisolating membranes. **(A)** The direct donor antigen presentation to host T cells is blocked by immunoisolating membranes. However, islet grafts produce DAMPs, which activate immune cells such as T cells and macrophages. Attracted macrophages and fibroblasts will cause pericapsular fibrotic overgrowth, which leads to hypoxia of the enveloped cells. **(B)** In case of application of xenografts, T cells might be stimulated also by indirect antigen presentation. If islet-derived xeno-epitopes go through the membrane and are presented via the MHC of host APCs to TCR, activated T cells might produce proinflammatory cytokines, which might give functional and structural damage to islet grafts. Membranes for xenografts do have to meet other requirements than for allografts. DAMP, damage-associated molecular pattern; MHC, major histocompatibility complex; APC, antigen-presenting cell; TCR, T cell receptor. Color images are available online.

novel immunomodulatory strategies that can be used in combination with encapsulated islets and describe the recent outcomes.

Regulatory T cells

Regulatory T (Treg) cells play a critical role in regulating immune reactions and in maintaining unresponsiveness to self-antigen, the state of immune tolerance.[94,95] These cells can be used to induce immune tolerance for donor antigens to avoid that the immune system destroys transplanted grafts and do this specifically without inhibiting the capacity of the immune system from fighting other antigens present on bacteria or viruses.[96]

Treg expansion was applied by Hu *et al.* to prolong the survival of human islet allografts in humanized diabetic mice.[97] A temporary treatment of 3 weeks with low-dose interleukin-2 combined with rapamycin significantly prolonged graft survival. They demonstrated that this was associated with expansion of Treg cells and anti-inflammatory cytokine production. Also, systemic infusion of Treg cells improved allograft survival, which could be further enhanced by local administration of Treg cells.[98,99] These studies revealed that systemic infusion of Treg cells is less effective than local administration because systemically administered Treg cells cannot fully migrate toward the islet graft. It was also reported that an agarose device can recruit Treg cells locally at the subcutaneous site and contribute to acceptance of the allograft.[100,101]

To make use of the immunoregulating properties of Treg cells in immunoisolation, Treg cells were conjugated to alginate and PEG-based polymer capsules. The Treg-conjugated capsule induced long-term normoglycemia in 60% of allogenic mouse recipients.[102]

Cotransplantation of immunomodulatory cells

Another approach is the application of cells with immunomodulating properties such as Sertoli cells and mesenchymal stem cells (MSCs). Sertoli cells can be found in the testicles and are known to generate an immunosuppressive environment.[103,104] MSCs are another immunomodulating cell type and can be found and isolated from, for example, bone marrow and adipose tissue.[105] They are known to produce anti-inflammatory cytokines and suppress immune reactions. Some rodent studies have demonstrated that co-transplantation of islets with MSCs promotes islet transplantation outcomes.[106,107]

Coencapsulation and transplantation of both Sertoli cells and MSCs showed a positive effect on graft survival. In a rat-to-mouse xenotransplantation model, 40% of mouse recipients of alginate microcapsules containing islets and Sertoli cells demonstrated normoglycemia 100 days after transplantation.[108] Similar beneficial effects have been observed for MSCs. Inclusion of MSCs in islet-containing capsules enhanced functional survival of syngeneic mouse islet grafts in 71% of the recipients, while only 16% of the mouse recipients became normoglycemic with islets alone.[109] Furthermore, coencapsulation of MSCs and islets in alginate-based microcapsules significantly reduced the FBR to the capsules in an allotransplantation model.[110] This mechanism was probably due to upregulated production of anti-inflammatory cytokines in the vicinity of the grafts.[110] Although longer term studies are needed, the current studies suggest that coencapsulation with these cell types and islets has beneficial effects and protects the islet graft from host responses.

Immunomodulation with apoptotic donor leukocytes

Apoptotic donor leukocyte (ADL) treatment is a novel promising approach for immunomodulation. Recipients receive intravenous infusion of apoptotic donor splenocytes before transplantation. The treatment induces alloantigen-specific tolerance and protects islet grafts from rejection in mouse models.[111] Notably, Singh *et al.* showed that pretreatment of ADL infusions together with short-term immunotherapy could protect allogeneic islet graft in five macaques over 1 year without encapsulation of the grafts.[112] ADL infusions can expand antigen-specific regulatory immune cell populations, including Treg cells, and suppress the expansion of effector cells, resulting in a stable donor-specific immune tolerance even in an NHP model.[112] Currently this approach has not been tested in combination with an encapsulation strategy or with xenografts, but holds significant promises for the future application of immunoisolated xenografts.

CXCL12 in combination with microencapsulation

CXCL12, which is known as an anti-inflammatory chemokine, has been reported to modulate immune responses by enhancing the polarization of T cells toward Treg cells and has been shown to inhibit the onset of autoimmune diseases.[113]

Chen *et al.*[114] showed that the incorporation of CXCL12 in alginate microcapsules significantly improved graft survival compared with encapsulated islets without CXCL12. Notably, incorporating CXCL12 protected also from graft failure in xenotransplantation.[114] Porcine islets encapsulated in alginate containing CXCL12 maintained normoglycemia in the recipient mice for a significantly longer period than encapsulated islets without CXCL12. CXCL12 selectively recruited Treg cells to the islet graft and thereby promoted graft survival. Furthermore, CXCL12 has been shown to attenuate the FBR against polymeric capsules in NHPs. The impact, however, on long-term survival of cynomolgus monkey islets remains to be demonstrated as encapsulated auto-islet grafts showed limitations in function 6 months after transplantation.[115]

CXCL12 not only seems to be beneficial for islets but also for acceptance of stem cell-derived β cells, which is a promising alternative for islets taking into account the current donor shortage. Human stem cell-derived β cells in alginate-based microcapsules with CXCL12 were transplanted into immunocompetent diabetic mice.[116] This inclusion of CXCL12 significantly reduced PFO and thereby enhanced long-term functional graft survival (>150 days) without systemic immunosuppression. Human C-peptide was detectable up to 20 weeks after transplantation and the levels were significantly higher than in the absence of CXCL12.

Immunomodulation with mAbs

Administration of mAbs that selectively target molecules associated with immune reactions can prolong graft

survival of various organs and tissues.[117] For example, Lee *et al.* demonstrated that inhibiting the CD154-CD40 signal by short-course administration of anti-CD154 mAb prolonged allogeneic mice islet graft survival to more than 250 days after transplantation.[118] The graft acceptance might be facilitated by generation of Treg cells. As the anti-CD154 mAb caused major side effects when it was used in humans,[119] the blockade with an anti-CD40 mAb has also been investigated.[120] Anti-CD40 prolonged graft survival in an NHP model but failed to inhibit humoral allosensitization, which is considered to be a possible reason for graft failure.[121] The combination of CTLA4-Ig with anti-CD40 mAb achieved graft acceptance and also prevented humoral allosensitization.[122]

Coencapsulation of porcine islets with CXCL12 and administration of the anti-CD154 mAb and CTLA4-Ig prolonged xenograft survival in autoimmune diabetic NOD mice.[123] They also found that coencapsulation of CXCL12 in combination with the anti-CD40 mAb, YTS177.9, was as effective as anti-CD154 mAb and CTLA4-Ig treatment in prolonging porcine islet survival in NOD mice. In another study, mouse islet encapsulation by conformal PEG coating was combined with short-term administration of anti-LFA-1 mAb. This had a synergistic effect and induced long-term normoglycemia (>100 days) in 78% of the recipients with the combination treatment. Without anti-LFA-1, 60% showed long-term survival.[124] It was found that the combination treatment decreased immune cell recruitment to the grafts, downregulated proinflammatory cytokines, and enhanced macrophage polarization into an immunosuppressive M2 phenotype, inducing Treg cells in the periphery.[124]

Site-specific delivery of immunomodulatory reagents

Site-specific delivery of immunosuppressive drugs is based on the supply of therapeutic agents at a specific site to avoid systemic toxicity.[125] Several groups have focused on developing drug carriers made of biomaterials. These devices carry, for example, anti-inflammatory agents and immunosuppressive drugs such as tacrolimus, rapamycin, or fingolimod, and were implanted in nearby islet grafts.[126–128]

Success of this approach has been shown with different drugs. Codelivery of islet grafts and tacrolimus-loaded microspheres could significantly improve graft survival of a rat-to-mouse model.[126] Sixty percent of the recipients that received tacrolimus-loaded particles maintained normoglycemia during the 30-day study period, while none of recipients with islets alone showed normoglycemia. Also, rapamycin-loaded microparticles and islet grafts were implanted together at the anterior chamber of the eye and delayed allogeneic mice islet graft rejection.[127] These studies suggest that these approaches seem to reduce rejection.

Other groups investigated the addition of local immunomodulatory reagents at the surface or in immunoisolating capsules. Park *et al.* used a rapamycin-PEG mixture to coat alginate microcapsules.[129] They implanted encapsulated porcine islets into the diabetic immunocompetent mice and the coating significantly reduced PFO. Both groups induced normoglycemia for 30 days, but there were no significant differences in graft survival between the groups. Farah *et al.* achieved long-term local controlled-release of GW2580, an inhibitor of colony stimulating factor 1 receptor, from alginate-based microcapsules and demonstrated that incorporation of crystalline GW2580 in the microcapsules significantly reduced FBR against the microcapsules for at least 6 months and over 1 year in NHP and rodent models, respectively.[130] The crystalline GW2580 inclusion showed long-term rat islet graft survival transplanted into diabetic immunocompetent mice. In addition, human islets in microcapsules with crystalline GW2580 could maintain their function up to 72 days post-transplantation in diabetic immunocompetent mice.

Immunomodulating polysaccharides on microcapsules

Another new approach is to include immunomodulatory molecules on the surface of capsules to support graft longevity.[30] Success of this approach was recently shown by incorporation of a specific type of pectin consisting of α-1,4-linked-D-galacturonic acid chains partially with methyl ester in the surface of microcapsules. This pectin molecule had the unique ability to attenuate immune activation via the TLR2-1 pathway, which recognizes DAMPs and causes proinflammatory responses.[81,131] This prolonged rat-to-mouse xenograft survival[90] and lowered proinflammatory cytokine responses in the intraperitoneal transplantation site. It was shown that the pectin-modified alginate capsules suppressed TLR2-1 signaling-mediated immune responses by competitive binding of proinflammatory molecules such as DAMPs with TLR2. The strategy might be a relatively simple approach for local immune modulation. The same pectin also protected islet cells from oxidative and inflammatory stress and by that might have dualistic effects.[132]

Conclusion

The number of T1D patients is still yearly increasing and over a million children have been diagnosed with T1D in 2019.[133] Islet transplantation has shown promising results for glycemic control in T1D, but is still applied at a limited scale due to factors such as the need for lifelong administration of immunosuppressive drugs and the shortage of insulin-producing cells.

This review focused on the work over the past decades on the different strategies that have been developed to allow transplantation in the absence of immunosuppression. Here we reviewed recent progress in encapsulation and the means to combine immunoisolation and immunomodulation to prolong survival of grafts and avoid loss of islet cells especially in the immediate period after implantation. Better ways to immunoprotect islets are emerging as replenishable sources for insulin-producing cells, such as xenografts and insulin-producing cells derived from stem or progenitor cells, will be available in the near future. There is an urgent need to develop methods to protect these grafts from rejection or the autoimmune processes in T1D recipients.

As discussed in the present review, immunoisolation has shown safety and feasibility, especially when using alginate, but it is still necessary to improve the encapsulated graft survival time by minimizing the immunogenicity of encapsulation polymers and reducing the influence of immune responses stimulated by islet grafts themselves. Several studies show that encapsulated islets evoke immune responses by release of DAMPs, but also, in case of xenografts, by indirect antigen presentation. Immunomodulation

strategies might be helpful for temporarily or permanently regulating these responses, which are associated with significant loss of islets in the immediate period after implantation. Current insight indicates that when applying xenotransplantation, additional treatment to attenuate the immune response might be needed to neutralize the proinflammatory cytokine production activated by the indirect antigen recognition.[85] Therefore, for xenotransplantation, combined strategies of immunoisolation and immunomodulation may be a solution.

Especially, a promising example of combining these two strategies is the local application of immunomodulatory chemokines or other molecules such as CXCL12, pectin, and GW2580 that provide an immunosuppressive microenvironment around the transplanted microencapsulated islets and enhance graft survival times.[90,114,134] Notably, CXCL12 improved functional survival of human stem cell-derived β cells in a mouse model and reduced FBR in an NHP model.[115,116] However, further research demonstrating efficacy in larger animal models and subsequent clinical trials are needed.

In summary, recent clinical researches have demonstrated the safety and feasibility of immunoisolation strategies for diabetes treatment, but there is still a need for efficacy studies. There is also an urgent need to develop strategies for enhancing the longevity of grafts. The current insight is that loss of islet cells in the immediate period after transplantation is one of the factors leading to reduced graft survival times and that combining immunoisolation and immunomodulation might prevent this and improve encapsulated islet cell graft survival. This combined approach might be essential for the future of islet transplantation and our perspective may contribute to establish a novel therapeutic application of encapsulated islets to treat diabetes.

Authors' Contributions

All authors contributed to the writing and editing of the article.

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