nature reviews endocrinology

https://doi.org/10.1038/s41574-024-01029-0

Review article

Check for updates

Harnessing cellular therapeutics for type 1 diabetes mellitus: progress, challenges, and the road ahead

A list of authors and their affiliations appears at the end of the paper

Abstract

Type 1 diabetes mellitus (T1DM) is a growing global health concern that affects approximately 8.5 million individuals worldwide. T1DM is characterized by an autoimmune destruction of pancreatic β cells, leading to a disruption in glucose homeostasis. Therapeutic intervention for T1DM requires a complex regimen of glycaemic monitoring and the administration of exogenous insulin to regulate blood glucose levels. Advances in continuous glucose monitoring and algorithm-driven insulin delivery devices have improved the quality of life of patients. Despite this, mimicking islet function and complex physiological feedback remains challenging. Pancreatic islet transplantation represents a potential functional cure for T1DM but is hindered by donor scarcity, variability in harvested cells, aggressive immunosuppressive regimens and suboptimal clinical outcomes. Current research is directed towards generating alternative cell sources, improving transplantation methods, and enhancing cell survival without chronic immunosuppression. This Review maps the progress in cell replacement therapies for T1DM and outlines the remaining challenges and future directions. We explore the state-of-the-art strategies for generating replenishable β cells, cell delivery technologies and local targeted immune modulation. Finally, we highlight relevant animal models and the regulatory aspects for advancing these technologies towards clinical deployment.

Sections

e-mail: agrattoni@houstonmethodist.org; jgiraldo@breakthrought1d.org; mark_poznansky@dfci.harvard.edu; p.de.vos@umcg.nl

Key points

- Stem cell-derived islets have advanced as a viable renewable source of cells for transplantation in type 1 diabetes mellitus (T1DM). Although these cells are being tested in the clinical setting, challenges remain to be addressed regarding cell safety, composition and function.
- Genetic engineering of renewable β cells can reduce immunogenicity, lower metabolic needs and bolster hypoxia resistance. However, the effect on β cell performance requires further elucidation.
- Local immunomodulation via in situ delivery of immunomodulatory molecules and adjuvant cells is emerging as a promising approach for abrogating the need for systemic immunosuppression in β cell transplantation.
- Current preclinical results suggest that immunoprotected islet cell grafts in a retrievable subcutaneous site could restore normoglycaemia for at least 1 year or longer without systemic immunosuppression.
- Despite the potential of new technologies, the development of cell therapy treatments must pragmatically focus on generating therapies that are not only effective and safe but also align with the real-world dynamics of patients' lives and the capabilities of health-care systems.

Introduction

Type 1 diabetes mellitus (T1DM) is an autoimmune disease affecting 8.75 million people worldwide, of whom ~1.52 million are <20 years old[1]. According to the US Centers for Disease Control and Prevention, 1.7 million adults (>20 years old) and 304,000 children and adolescents (<20 years old) had diagnosed T1DM in the USA in 2021 (ref. 2). The prevalence of this disease is predicted to grow in the next few decades[3]. In T1DM, the progressive immune-mediated destruction of pancreatic β cells leads to insulin insufficiency and chronic hyperglycaemia[4]. Stringent glucose control via continuous or episodic blood glucose monitoring and commensurate insulin dosing mitigates T1DM complications to some extent, but individuals affected by T1DM remain at elevated risk of hyperglycaemia and hypoglycaemia events. Technological innovations such as continuous glucose sensing and smart infusion pumps alleviate this burden for patients[5]. However, mimicking the β cells' intricate biological feedback loop and physiological insulin kinetics remains challenging, and patients struggle to achieve glycaemic control targets[3,6]. Pancreatic islet or β cell replacement represents a potential functional cure for T1DM[7–9]. Usually, pancreatic islets are transplanted into the liver via infusion through the portal vein. Upon transplantation, these cells have the potential to reverse T1DM in patients by restoring glycaemic control. The procedure is FDA-approved for the transplantation of allogeneic islets.

Although a small subset of the patient population has benefited from islet transplantation, the method of cell delivery could be improved and made accessible to more individuals with T1DM by addressing challenges such as cell dispersion, poor engraftment and reduced cell survival[10]. The limited availability of donor pancreata and variations in the quality and quantity of harvested islet cells also affect the widespread applicability of this procedure. Furthermore, immune rejection requires lifelong systemic immunosuppressive regimens, which expose patients to heightened risks of opportunistic bacterial and viral infections[11], and neoplasms[12]. These challenges have prompted the exploration of alternative and replenishable cell sources, surgically accessible transplantation sites, and approaches to enhance the engraftment, survival and function of transplanted cells, while obviating the need for chronic systemic immunosuppression[13].

Here, we provide a comprehensive overview of the state of the art of cell transplantation for T1DM. Specifically, this Review delves into current protocols for generating replenishable β cell sources. Next, we discuss cell delivery technologies and present an overview of advanced approaches for immune modulation and the generation of immune tolerance. Additionally, we examine the most relevant animal models used in the field. We discuss clinical translation pathways for β cells and delivery systems to provide insight into the regulatory pathway and standing challenges for the clinical deployment of these products. Finally, we present the outlook of the remaining challenges and future developments in the field.

Renewable islet cell sources

The limited availability of islets and reliance on sourcing this tissue from deceased donors has long hindered the progress of cell therapy for T1DM. In vitro differentiation of human pluripotent stem cells (hPSCs) into islet-like clusters is a viable method for generating β cells from a renewable source that addresses this challenge.

Stem cell-derived islets are generated by using combinations of factors sequentially to produce developmental intermediates, before inducing pancreatic endocrine and insulin-producing β cells[14,15] (Fig. 1). The final stem cell-derived islet composition consists of an endocrine-enriched population of cell types resembling β, α and δ cells. Following transplantation and a period of in vivo maturation, these cells can respond to various secretagogues and are capable of glucose-stimulated insulin secretion and control of blood glucose in multiple mouse models of diabetes. These attributes make stem cell-derived islets attractive for use in the clinic. Vertex Pharmaceuticals is currently conducting a clinical trial with a stem cell-derived islet product called VX-880 (ref. 16). Although the findings have not been peer-reviewed, they report that six patients with T1DM and severe hypoglycaemic unawareness have received these cells, and all have demonstrated insulin production and improved glycaemic control, including reduced levels of HbA_{1c} and increased time during which blood glucose is within the normal range, while reducing or eliminating the need for exogenous insulin[17,18]. In addition, two patients fulfilled the criteria for the primary goal, which involved the eradication of severe hypoglycaemic events and maintaining an HbA_{1c} level of <7%.

Despite rapid scientific progress in stem cell-derived islet technology, challenges remain for the use of these cells as a drug product for T1DM therapy[19]. Stem cell-derived islets are not equal to primary pancreatic islets functionally, transcriptionally or epigenetically, showing lower insulin secretion, immature transcriptional identity and inappropriate chromatin accessibility for both on-target and off-target genomic regions[14,15,20–22]. Fortunately, mouse models have shown that long-term transplantation improves stem cell-derived islets in all these areas[20,23,24]. However, methods to enhance the function and identity of stem cell-derived islets during the in vitro production process to levels seen after transplantation remain to be determined. Another challenge is that it is not possible to control the ratio of β, α and δ cells with current methods. During in vitro differentiation, a substantial number of cells tend to acquire an identity that produces serotonin so that they resemble enterochromaffin cells[22], and are therefore unlikely to be helpful for T1DM cell therapy. Surface markers have been identified

enabling on-target cell types to be purified[22,25,26], which might improve the functional potency and safety of the stem cell-derived islet drug product, but this approach might be difficult to scale up for manufacturing. Regardless, the optimal composition of cell types to maximize utility in T1DM cellular therapy is currently unknown.

The path towards developing large-scale and cost-efficient biomanufacturing of stem cell-derived islets to treat a substantial number of patients is challenging. Although a few million stem cell-derived islet cells can restore glycaemic control in a mouse model of diabetes[27], ~10^9 cells per dose will probably be necessary to treat adult human patients[28,29]. This estimate is based on clinical work with cadaveric human islets, and dosing must be confirmed in clinical trials for each stem cell-derived islet cell product. It is possible to produce stem cell-derived islets by propagating and differentiating hPSCs entirely in suspension[14], which typically achieves a density of 10^6 cells/ml by scaling in three dimensions. In the biotechnology industry, large-volume bioreactors are frequently used to scale up the production of monoclonal antibodies. However, it is unclear whether stem cell-derived islet manufacturing could be achieved in bioreactors with a volume of >1,000 l, as mechanical forces, such as those caused by convection necessary to prevent adhesion and sedimentation, negatively affect growth, survival and differentiation of hPSCs[30,31]. Alternatively, shear stress could be avoided by culturing and differentiating hPSCs on adherent cultures followed by aggregation into islet-like structures[32]. However, this two-dimensional approach intrinsically limits the scale of production. Automation is set to enhance scalable manufacturing for suspension and adherent systems, while simultaneously decreasing variability[33].

Regardless of the method used for cell manufacturing, comprehensive characterization of the final cell population is required to guarantee the safety of the product. Residual uncommitted cell types, such as hPSCs, within the final cell population could form undesired overgrowths or tumours upon transplantation[34,35]. In addition, lack of genomic stability and accumulation of genetic variants in oncogenes can be acquired in time and pose a safety risk[36]. For example, therapeutic cell mutations led to the suspension of a macular degeneration clinical trial in Japan[37]. Karyotypic abnormalities[38], variants in *TP53* (ref. 39) and variants in *BCOR*[40], which are all associated with various cancers, have been observed in hPSCs[41–43].

In summary, along with the development and optimization of cell manufacturing techniques, standard characterization protocols of cell products capable of determining composition, potency, and genomic status will be critical for the clinical adoption of these technologies and for ensuring patient safety[44–46]. In addition, clinical deployment will hinge on effective cryopreservation for storage, distribution and cost-effectiveness to make the therapy accessible and sustainable.

Cell delivery strategies

Conventional pancreatic islet transplantation via the Edmonton protocol has shown promise in restoring exogenous insulin independence in patients with T1DM. The success of the procedure hinges on key factors, including preservation of islets from hypoxic injury during isolation and subsequent handling (discussed elsewhere[47,48]), and systemic immunosuppression upon transplantation to avoid rejection. Additional factors, such as immediate blood-mediated inflammatory response, cell hypoxia in vivo, dispersion and exhaustion[49], are also relevant. Cell microencapsulation and macroencapsulation devices using semipermeable materials as a physical barrier have been explored as a strategy to deliver cells and abrogate immune rejection while eliminating the need for systemic immunosuppression. Other delivery systems that have been explored, such as open devices, scaffolds and hydrogels, permit the infiltration of blood vessels to directly vascularize and fully integrate the cell graft with the host.

Microencapsulation for immune isolation

In microencapsulation, cells are enclosed in gel-like microspheres that average <1,000 µm in diameter, many of which are implanted to deliver a therapeutic dose. Microcapsules contain a nanoporous barrier that protects transplanted cells from immune attack, while their small size and optimal surface-to-volume ratio allows fast exchange of glucose, insulin, nutrients and waste materials (Fig. 2). Among numerous materials developed for pancreatic islet microcapsules, alginate stands out for its excellent biocompatibility and adaptable chemical characteristics, which permit the encapsulation of delicate cell clusters, such as islets, through mild processing conditions[50]. The design of microcapsules, including their material, shape, porosity and surface properties, is critical to achieve long-term cell viability. Adjusting these factors along with applying functional coatings helps to control molecular transport and mitigate inflammation. However, challenges, such as the influx of inflammatory cytokines and release of damage-associated molecular pattern molecules (DAMPs) and antigens, can still trigger immune responses[51]. Additionally, pericapsular fibrotic overgrowth can impair the long-term viability of these systems by limiting molecular exchange, thus reducing oxygen and nutrient availability[52,53]. The success of microencapsulation also hinges on the site of implantation,

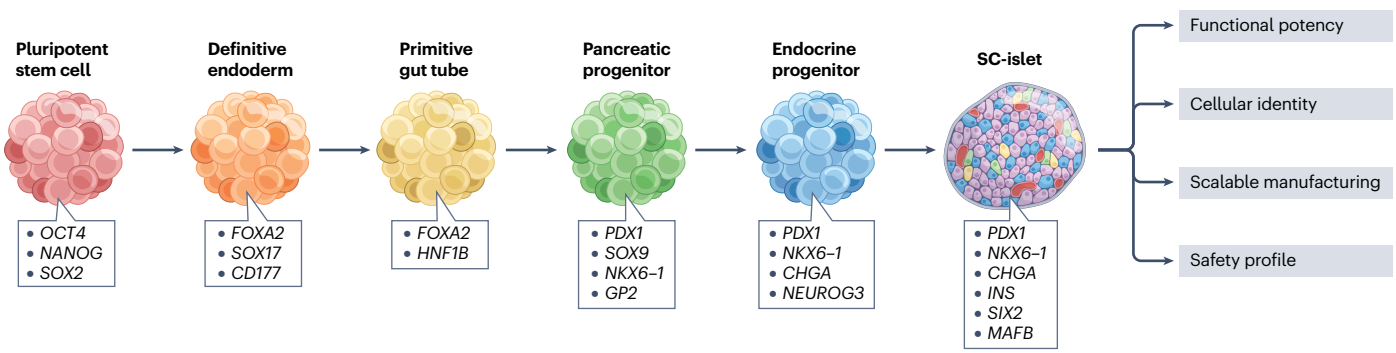


Fig. 1 | Pluripotent stem cell differentiation into stem cell-derived islets through modulation of different genes. Stem cell-derived islets (SC-islets) are functionally, transcriptionally and epigenetically different to native pancreatic islets. Enhancing cellular identity to generate optimal functional potency and safety profile is an active area of investigation along with methods for scalable manufacturing.

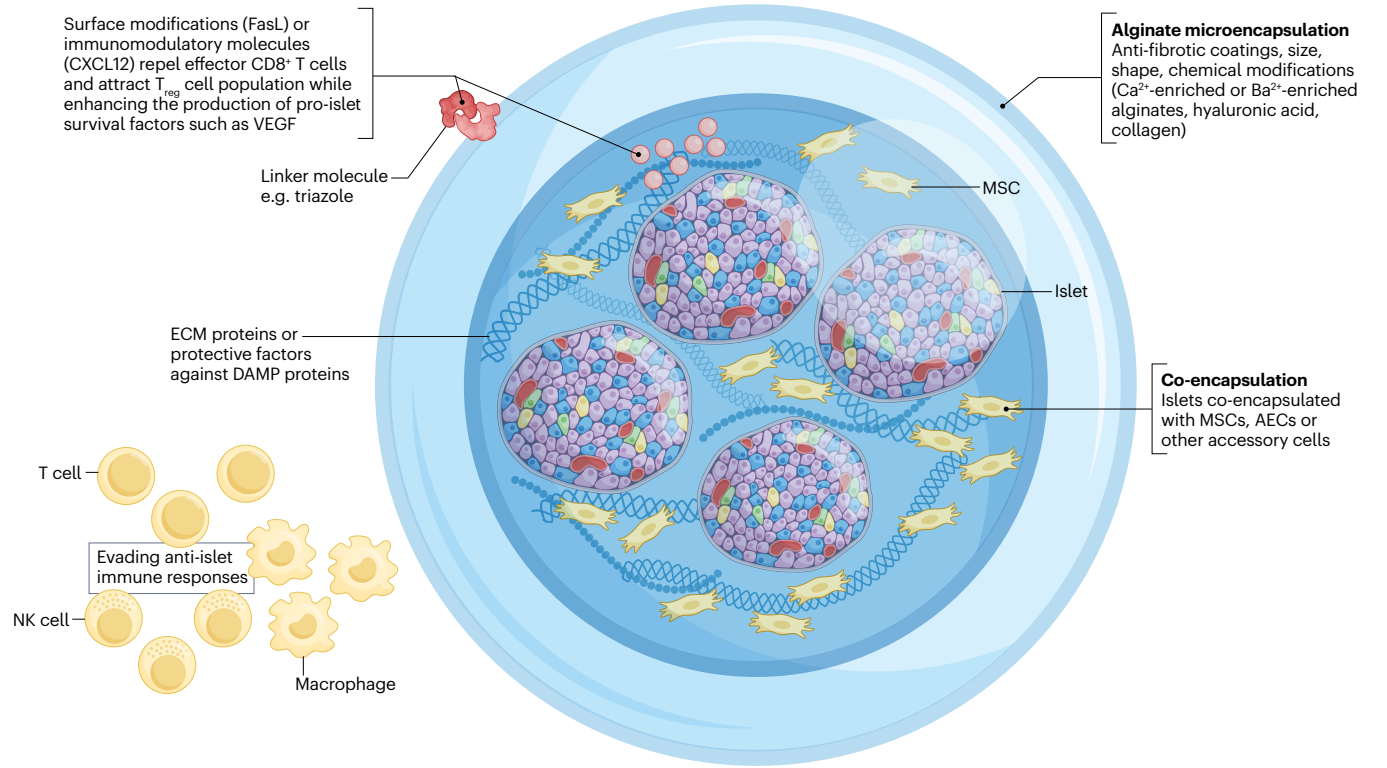


Fig. 2 | Microencapsulation approaches for islet transplantation. Microencapsulation strategies (most notably alginate with modifications including hyaluronic acid or collagen) for islet transplantation. Surface modifications and immunomodulatory molecules can promote islet survival. Co-encapsulation of immune modulatory cells (including mesenchymal stem cells (MSCs), amniotic epithelial cells (AECs) and other accessory cells and factors) as well as extracellular matrix (ECM) proteins can aid immune isolation and islet survival. DAMP, damage-associated molecular pattern; NK cell, natural killer cell; T_{reg} cell, regulatory T cell.

with considerations for accessibility, injection volume, blood supply and oxygen availability representing key factors.

Due to these challenges, although clinical trials have demonstrated the safety of alginate-encapsulated porcine islets[54], their long-term efficacy remains to be shown. To counter these issues, strategies include modifying the encapsulation material chemistry by including anti-fibrotic coatings and co-encapsulating islets with extracellular matrix (ECM). When transplanted into vascular and oxygen-rich sites such as the intraperitoneal cavity or omentum, islets microencapsulated in these materials[55,56] (Fig. 2) have shown prolonged survival for up to 1 year in mice[57], with no substantial foreign body response (FBR) and no loss of viability. In other studies, alginate combined with hyaluronic acid, a component of the ECM, increased islet viability and insulin secretion compared with alginate alone[58]. The addition of collagen, another ECM component, to hyaluronic acid led to further incremental improvements in islet viability in rat models[59]. Different islet sources might require specific alginate–ECM combinations to maximize longevity and function[60].

Incorporation of CXCL12, an islet-protective chemokine, in alginate microencapsulation supports the long-term function and immune protection of islet allografts, xenografts and human stem cell-derived islets in murine models of T1DM. Furthermore, preliminary transplantation studies in the omental sac of non-human primates (NHPs) have shown improvements in short-term xeno-islet survival and function and abrogation of FBR[61–63]. In other approaches, different materials have been used, including hyaluronic acid, chitosan, collagen, poly-L-lysine, and acrylic acid and their derivatives[64]. For example, poly(lactic-coglycolic acid) microspheres within islet microcapsules sustain the release of exenatide supporting transplanted cell viability[65]. Furthermore, co-encapsulation with adjuvant or accessory cells, such as mesenchymal stem cells (MSCs), known for their ability to promote tissue repair and angiogenesis and modulate immune response, has shown increased viability and less fibrotic growth[66,67] compared to alginate alone.

Two challenges remain for the use of microcapsules: one, ensuring adequate mass transfer (that is, oxygen, glucose and insulin) between the intracapsular space and surrounding environment; and two, managing the graft's overall size. Microcapsules larger than approximately 400 μm in diameter require substances within the islet to diffuse over a greater distance and a larger graft volume. Moreover, capsule agglomeration due to settling in bipedal animals can exacerbate challenges with poor diffusion and nutrient delivery. To this end, conformal islet coatings with thin layers of immune-isolating hydrogels have been developed to reduce both diffusion distance and graft volume, and have shown promise in β cell replacement therapies in mouse and NHP models of T1DM[68–70].

New methods facilitate the production of smaller capsules, which are suitable for transplantation into scaffolds (see the section 'Open devices and scaffolds'). Embedding into a scaffold enhances the

potential for graft retrieval or replacement and has the potential to broaden the reach of transplantation to more individuals with T1DM.

Macroencapsulation for immune isolation

Macrocapsules are designed to deliver large doses of cells in a retrievable unit. Device size, geometry and placement are crucial design considerations. An ideal site for transplantation must include a dense vascular network to provide adequate oxygen and nutrient supply and support insulin and glucose exchange, as well as a suitable microenvironment to minimize cell loss after transplantation. Moreover, it should facilitate a minimally invasive surgical procedure for implantation, monitoring and retrieval[71]. All of these considerations are fundamental to achieving sufficient cell viability, device function and the clinical relevance of the approach.

To address these design challenges, a broad range of macrocapsule devices have been developed, including hollow-fibre (intravascular or extravascular)[72,73], planar[15,74,75] and cylindrical structures[76], with advances in 3D printing and microfabrication leading to more complex designs[77,78]. However, due to FBR[79] to the implant, all these devices encounter fibrosis, which impairs the essential exchange of oxygen, nutrients, glucose and insulin that is crucial for cell viability and function. To mitigate fibrosis, different approaches, including chemical modifications, surface topology design and local drug release, have been reported[80].

The large surface-to-volume ratio of macrocapsules can also restrict the diffusion of oxygen and nutrients to the encapsulated cells. It is now accepted that the partial pressure of oxygen within typical implantation sites (subcutaneous or intraperitoneal) is not able to support the viability and function of islets at the high cell density required for a practical implant footprint[81–83]. This understanding has led to the development of various systems to supplement oxygen within macroencapsulation devices in vivo, the advantages and limitations of which are reviewed elsewhere[83,84]. Overall, in vivo preclinical studies evaluating these oxygen supplementation approaches have shown benefits to the encapsulated cells. However, clinical proof-of-concept studies with the βAir device[75,85] provided mixed results, suggesting that while oxygen supplementation may be necessary, optimized delivery regimens remain to be developed to consistently improve clinical outcomes.

ECM[86], accessory cells and other biofactors co-encapsulated with islets have also been explored to support cell viability and function in macrocapsules. In a clinical study using co-transplantation of porcine islets and Sertoli cells in prevascularized hollow tubes, five of 12 non-immunosuppressed adolescents showed reduced insulin requirement, and one achieved insulin independence[87,88]. In a preclinical study, porcine islets co-encapsulated with a collagen matrix within a subcutaneous planar alginate device successfully improved diabetes control (HbA_{1c} <7%) in NHPs for 6 months without immunosuppression[89]. In another study in NHPs, porcine islets co-encapsulated in a structure with adipose-derived MSCs maintained glycaemic control for >32 weeks[90]. Finally, subcutaneous transplantation of a macroencapsulation device equipped with zero-order release of alanine and glutamine led to a 30% increase in cell survival in mice[91]. However, demonstration of efficacy of a scaled-up device in clinical studies remains elusive, probably because of the increased diffusion distances and stronger fibrotic responses.

The complex nature of macroencapsulation devices, which includes cells, materials and other components, has cost implications for translation. Further, the complexity complicates the regulatory pathway, which involves simultaneous oversight by multiple regulatory bodies, each evaluating different aspects of these technologies[92]. However, progress has been made by companies such as ViaCyte and Vertex[93,94]; in this context, the VX-264 phase I–II clinical trial[95] examining the safety, tolerability and efficacy of an immunoisolating device containing stem cell-derived islets may provide insights for further developments of immunoisolating macroencapsulation.

Open devices and scaffolds

In their native microenvironment, islets are densely vascularized via an intricate network of fenestrated capillaries that enables communication with the body via paracrine and endocrine signalling, facilitating a fine-tuned mechanism of sensing and responding to glucose, nutrients, hormones and other molecules[96]. Islet vasculature actively contributes to islet function by enhancing blood flow at increasing levels of circulating glucose and by regulating β cell activity[97] and proliferation[98].

Insufficient vascularization is widely acknowledged as a primary factor causing delayed and suboptimal glycaemic control after islet transplantation. Recognition of the interdependent relationship among islets, ECM, vasculature and innervation has driven the development of biomaterials and devices that facilitate direct host–graft vascularization and innervation[99]. These vascularization approaches can be categorized into two primary strategies: one, simultaneous implant–transplant in which islets are pre-embedded within the implant at the time of implantation; and two, prevascularization systems in which cell transplantation is performed days or weeks after initial implantation of the device and a prevascularization phase.

The choice of transplantation site is critical for the effective deployment of direct vascularization modalities. Considerations include factors such as vascular remodelling potential, oxygen supply, invasiveness of surgical procedures, device accessibility for manipulation, retrieval and imaging, and mimicking physiological insulin release and action (that is, portal uptake). For both vascularization strategies, the omental, intraperitoneal and subcutaneous spaces are viable sites for implantation. The omental space provides a well vascularized site, and the intraperitoneal space affords ample room, and both mimic the portal–peripheral insulin gradient present in physiological insulin release. However, both locations are less accessible and require more invasive surgical procedures than subcutaneous sites. Subcutaneous sites have limited vascular supply, but the use of pro-angiogenic factors and cells can mitigate this limitation[100].

Notably, direct vascularization exposes grafts to immune rejection, which is traditionally countered via chronic systemic immunosuppression. The severe adverse effects of these lifelong chronic systemic regimens have prompted the development of local immunomodulatory strategies, either immunosuppressive or to generate and maintain a tolerogenic microenvironment[101] (see the section ‘Alternative immunoprotection methods’). Additionally, strategies involving vascular anastomosis can subject transplanted cells to sudden high blood pressure, risking mechanical stress and microarchitectural disruption; however, in many direct vascularization systems, cells are vascularized progressively, thereby mitigating this risk.

Simultaneous implant–transplant approaches. In simultaneous implant–transplant strategies, islets are delivered within blood vessel-permeable and biodegradable matrices or open scaffolds (Fig. 3). These approaches are centred on forging vascular connections with the host via intrinsic material properties, the local delivery of pro-angiogenic growth factors and nutrients, and/or the co-delivery of pro-angiogenic

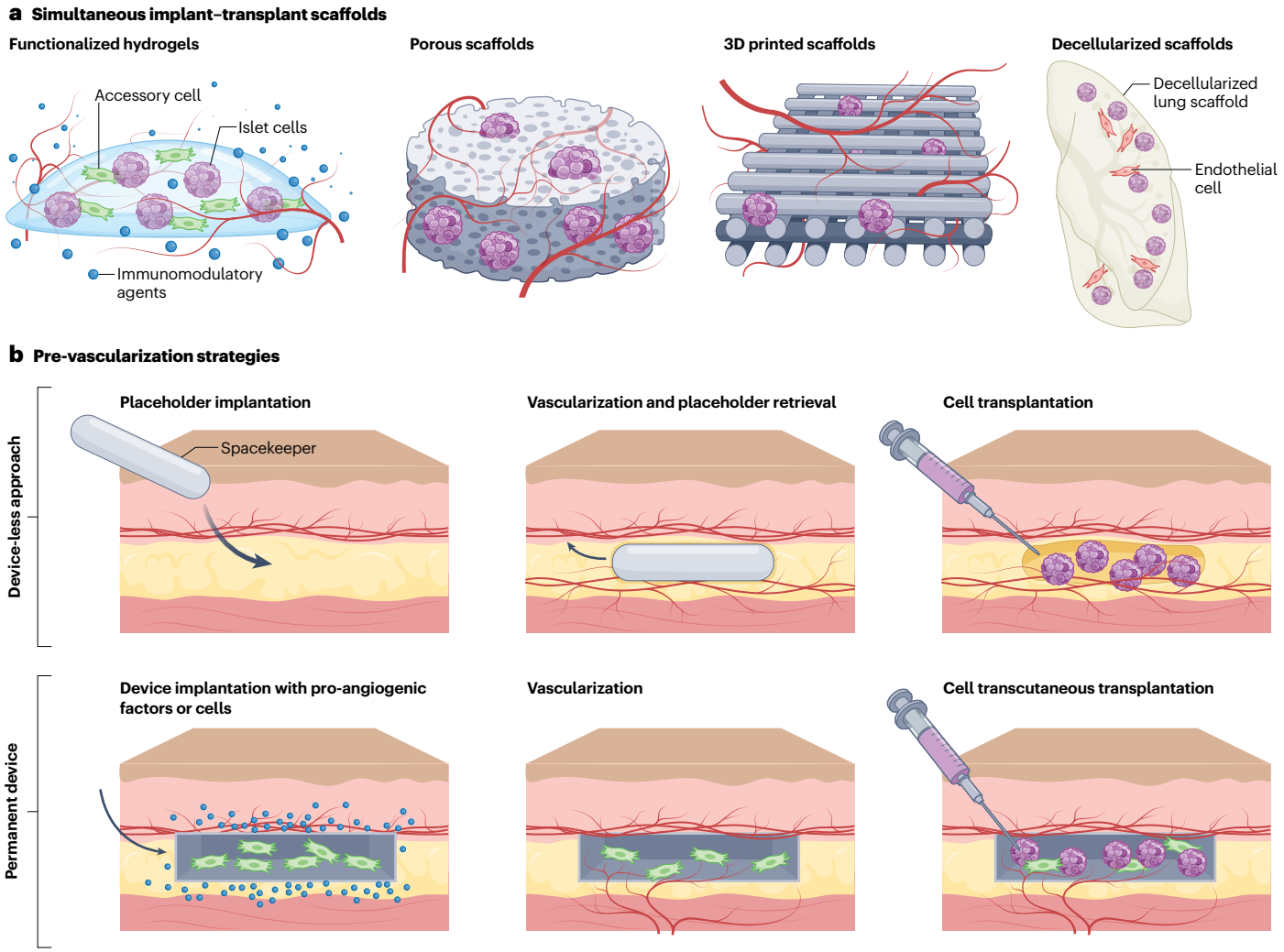


Fig. 3 | Direct vascularization scaffolds and open devices. a, Simultaneous implant–transplant methods involve placing islets in permeable matrices or open scaffolds, relying on the host's vascular connections. Approaches include embedding islets in matrices that deliver pro-angiogenic factors and accessory cells such as endothelial cells and mesenchymal stem cells in hydrogels or collagen matrices. Some methods utilize microvascular fragments for faster integration, while 3D printing creates structures that mimic natural tissue architecture. Decellularized tissues can also be used as scaffolds. **b**, Prevascularization techniques establish a vascular network prior to transplantation to improve outcomes. Both temporary and permanent devices are being explored, with some using methacrylic acid to reduce hypoxia and fibrosis, while minimizing immune response.

accessory cells (such as endothelial cells and MSCs)[102]. Examples of matrices demonstrating successful vascularization, engraftment and diabetes reversal in preclinical studies include vascularizing degradable methacrylic acid–polyethylene glycol and pH-optimized polyethylene glycol hydrogels[103,104], hydroxypropylmethyl cellulose hydrogel integrated with plasma components[56], and collagen, glutamine and serum mixture matrix[100].

A limitation of simultaneous implant–transplant approaches is the potential for developing hypoxia during the peri-transplant period prior to formation of new vascular networks. In fact, several of these methods are slow to achieve meaningful perfusion, with a considerable loss of islets within the first 24–48 h, and the vessels they produce are often transient or highly permeable[105]. To address this challenge, an innovative approach used microvascular fragments to speed up vascular integration of the graft[106,107] and glucose normalization, but the efficacy of human microvascular fragments, especially from patients with diabetes, remains to be evaluated.

Co-transplantation of accessory cells that support vascularization requires considerations about cell sourcing and regulatory barriers. Immunologically compatible, autologous primary vascular cells or microvessel fragments can pose translational difficulties related to sourcing, processing and implementation. By contrast, allogeneic cells are more amenable to translation, but their use faces challenges related to immune rejection.

Decellularized tissue scaffolds. Decellularized tissues serve as promising scaffolds for pancreatic islet transplantation due to their ability to retain endogenous ECM[108], which is pivotal in driving islet survival

and function[57,109], and their ability to support tissue remodelling and graft integration. Decellularized matrix is a crucial component in open scaffold engineering, particularly in the development of functional vascularized endocrine constructs. The combination of native organ architecture and ex vivo prevascularization has the potential to mimic the native endocrine environment prior to in vivo deployment[110]. For this, bioreactors allow tailored cell seeding and hierarchical perfusion, functionalities that facilitate the spontaneous self-assembly of the organ and foster the development of specialized functions[111]. For instance, decellularized lung scaffolds have been successfully repopulated with primary pancreatic islets or immature neonatal porcine islets, along with human endothelial cells[110,112].

As a result of this, the engineering process has enabled controlled islet engraftment within a vascularized ECM environment, leading to the attainment of ex vivo function under dynamic perfusion culture conditions. This innovative approach combines prevascularization strategies with islet engraftment ex vivo, offering the potential for successful peri-transplant scaffold engraftment coupled with rapid endocrine function restoration in vivo[24]. Additionally, this approach results in a testbed for various in vitro assessments of interest, such as monitoring the immunogenicity of cells, or the function of immunomodulatory agents in mixed lymphocyte assays.

3D-printed cell-laden architectures. Porous scaffolds fabricated using traditional particulate leaching techniques or additive manufacturing have long been used to both house and distribute islets within a 3D space[113]. These systems drive host tissue infiltration and vascularization while offering specific mechanical properties and geometrical features to house and protect transplanted cells and aid their engraftment. Successful long-term host integration and FBR to these 3D-printed porous systems are highly dependent on various parameters, including the physicochemical and mechanical properties of the material, device scale, porosity and material topography[80]. Various studies have demonstrated the capacity to print cells within ECM-based hydrogels to generate custom 3D engineered tissues with the potential to mimic the native pancreas anatomy[114]. 3D-printed biomaterials can also recapitulate ECM cues by encapsulating islets within decellularized pancreas derivatives or through customized ECM-inspired hydrogels[104,109]. These materials preserve islet function and improve stem cell-derived islet cell differentiation[77]. Like other direct vascularization platforms, 3D-printed islet devices show a lag between implantation and the formation of a competent intra-device vascular bed and/or host–graft vascular connections. Leveraging rational geometrical placement of cells, biomaterials and accessory components, various 3D printing approaches have been studied to temporarily provide oxygenation[115] and essential nutrients, or to accelerate vascular formation.

3D-printed implants have already demonstrated durable therapeutic efficacy in various preclinical models including rodents, pigs and NHPs[116]. In addition, the rapid evolution of additive manufacturing technologies offers new opportunities for the generation of hybrid biomaterial structures with tissue-mimetic properties and geometrical features that are ripe for new developments in β cell transplantation[19]. These biofabrication approaches can generate products in a more reproducible manner to deliver more consistent outcomes.

Prevascularization systems and devices. Prevascularization systems are designed to generate a patent vascular network before cell transplantation to promote rapid islet vascularization and engraftment, and minimize hypoxia and cell death during the peri-transplant period[117,118]. Achieving early engraftment is fundamental not only for rapidly achieving glucose homeostasis but also for abating the production of DAMPs, which can subsequently prime adaptive immune responses[119–121]. Prevascularization also decouples the trauma associated with biomaterial implantation and β cell delivery. Prevascularization can be accomplished via device-less or permanent device strategies[122–124] (Fig. 3). In device-less approaches, a temporarily placed biomaterial harnesses the natural inflammatory response, FBR, and vascular network deposition. Efficacy has been demonstrated for mouse and human[122] islets and for stem cell-derived islets[125,126]. One notable example of permanent devices is the Sernova Cell Pouch System, which has produced promising results in humans[127]. In strategies such as these, neovascularization can be augmented to enhance outcomes through coatings and hydrogels with pro-angiogenic materials, such as methacrylic acid[113], which may overcome hypoxia and the diffusion barrier presented by a fibrotic layer. However, the chemical and physical properties of the prevascularization strategy must be taken into consideration to minimize peri-implant FBR. The use of platelet-rich plasma[128] or co-transplantation of pro-angiogenic accessory cells, such as MSCs[128,129], or MSC-derived cell products (such as extracellular vesicles), endothelial cells, perivascular cells[102], microvessels[106] and vascularized islet organoids containing human amniotic epithelial cells and human umbilical vein endothelial cells[130], can circumvent the lack of rapid revascularization at the site of transplantation, and might support β cell replacement in extrahepatic, prevascularized sites. As stated above, co-transplantation of accessory cells that support vascularization requires considerations about cell sourcing and regulatory barriers.

Alternative immunoprotection methods

β cell replacement in patients with T1DM presents unique challenges in relation to other cell therapies for non-autoimmune diseases. Even with autologous stem cell-derived islet transplants derived from induced pluripotent stem cells (iPSCs), patients with T1DM would require strategies to prevent recurrence of autoimmunity. Current immunosuppressive therapies in islet allotransplantation involve induction (intense initial suppression), maintenance (long-term suppression to avoid chronic rejection) and treatment for acute rejection, all of which are systemic and non-specific, leading to complications such as lymphopenia, immunodeficiency, organ toxicity and damage[131], and heightened risks of infections and neoplasms. Moreover, some of these drugs can also impair islet function.

Strategies are emerging for more targeted and less toxic immunomodulation, including the use of biomaterials for localized, site-specific drug delivery, incorporation of immunomodulatory cells, and reducing the immunogenicity of transplanted β cells. Immunomodulatory induction of tolerance to the islet autoantigens and alloantigens requiring transient rather than chronic administration and yielding long-term effects would represent a true cure for T1DM following islet transplantation. Current clinical evidence suggests that tolerance may only result from bone marrow transplants that establish haematopoietic chimerism[132]. To date, even the most advanced targeted immunomodulation methods have achieved only operational tolerance.

Targeted immunomodulation

Various biological drugs are under investigation for the suppression of T cell activation, while aiming to minimize the severe side effects

Box 1 | Clinically relevant immunosuppressive and immunomodulatory agents

Achieving islet transplantation without systemic immunosuppression is a key goal in T1DM. The antigenicity and immunogenicity of transplanted islet cells determine the need and type of immunosuppression. Gene editing aims to create hypoimmune cell products requiring milder immunosuppressive regimens. The transplantation site also influences immune responses, which will become clearer as new sites beyond the traditional intra-portal route are explored.

Currently, T cell-depleting induction agents are commonly used, but the required T cell depletion level may change with different cell products and transplant sites. Modulating maintenance immunosuppression may allow lower levels of immunosuppressive agents, favouring tolerance-promoting drugs such as rapamycin over calcineurin inhibitors.

Selected centres in Europe prefer basiliximab, a monoclonal antibody targeting the IL-2 receptor on T cells, for induction therapy. Although well tolerated, these agents may lack potency. North American centres often favour thymoglobulin (rabbit antithymocyte globulin), which spares T_{reg} cells and supports low-dose protocols. Alemtuzumab (anti-CD52) has also been successfully used in clinical trials.

Anti-inflammatory strategies are also important during the peri-transplant period to minimize early graft loss. Agents such as etanercept (anti-TNF), anakinra (IL-1 receptor antagonist), and GLP1 receptor agonists have been explored and remain highly relevant. Tacrolimus is an effective maintenance immunotherapy but has adverse effects including increased infection risk and organ toxicity, leading to interest in calcineurin-free regimens. In this context, a novel protocol with anti-CD40L (costimulatory blockade) is being clinically tested. Beyond systemic approaches, local sustained delivery of immunomodulatory agents is the focus of numerous efforts. Local immunosuppression with agents such as CTLA4Ig, thymoglobulin, anti-CD40L, anti-IL-6 and anti-CD2 is under preclinical investigation. Further, future trials will explore local administration of FasL at the transplant site with transient systemic rapamycin.

Ultimately, eliminating chronic systemic immunosuppression will probably require a combination of strategies, including reducing cell immunogenicity, using local immunomodulation and tolerance induction.

and lack of specificity associated with conventional immunosuppressants (Box 1). These strategies include blocking co-stimulatory signals necessary for activation of autoreactive and alloreactive T cells either on antigen-presenting cells (CTLA4Ig) or on T cells (anti-CD40L), with promising results in clinical trials of kidney transplantation[133]. These strategies also aim to increase the number of regulatory T (T_{reg}) cells[132–143]. Furthermore, drugs that block innate immune cells (TNF, IL-1β and CXCR1/2) and promote induction of myeloid-derived suppressor cells are being evaluated as a combination strategy[144–147].

The small volume of functional islet grafts and their transplantation into confined sites uniquely enables the local co-delivery with immunomodulatory drugs. However, achieving adequate longevity of treatment remains a hurdle.

Site-specific immunomodulation via biomaterial-mediated drug delivery. Biomaterial approaches for localized immunomodulation include nanomaterials and nanoporous membranes, microgels and microcapsules, and scaffolds. Biomaterial–drug formulations for intradermal delivery can be tailored by modulating the size of the biomaterial carrier to achieve passive targeting of the graft-draining lymph nodes via lymphatic drainage and of inflamed sites via enhanced vascular permeability and retention effect. Nanomaterials can increase the solubility of hydrophobic drugs[148] and allow depot effects at target sites and in cells, and nanoformulations have been used to deliver traditional immunosuppressive drugs (for example, cyclosporine[148]), DAMPs and islet antigens[122,149]. However, their size causes rapid uptake by immune cells and impedes their use for delivering extracellular signals (such as PDL1 and CTLA4Ig). Conversely, larger microgels and microparticles are unable to target draining lymph nodes and can be retained at the graft site. These materials have been used to deliver either traditional immunosuppressive chemicals (steroids[150], tacrolimus[151–153] and rapamycin[154]), biological drugs (CTLA4Ig[155]) and chemokines (CXCL12 (ref. 156), IL-2 (ref. 157) and TGFβ1 (ref. 158)) or immunomodulatory factors (such as FasL[159] and PDL1 (ref. 160)) and antioxidants[161]. However, they are subjected to host innate immune responses that could lead to their phagocytosis and/or fibrotic encapsulation, decreasing their therapeutic efficacy. Intra-lymph node injection of microparticles delivering immunomodulatory drugs (for example, rapamycin) along with antigens is an alternative to co-delivery with islets to provide targeted immunomodulation in the graft-draining lymph nodes[162].

Site-specific immunomodulation via islet co-delivery with immunomodulatory cells. Localized immunomodulation can also be achieved by islet co-delivery with immunomodulatory cells, such as MSCs, which decrease inflammation, promote tissue regeneration[163–166] and improve islet survival[167]. To enhance their immunomodulatory properties, MSCs have been genetically modified to secrete immunomodulatory factors that can target T cell activation (for example, CTLA4Ig and PDL1 (ref. 168)) or co-delivered with drug-eluting biomaterials[169]. One limitation of MSCs is the limited permanence and duration of their immunomodulatory effects owing to their migratory behaviour[170]. MSC encapsulation might address this challenge and achieve local retention of MSC-secreted factors (such as exosomes and extracellular vesicles)[171,172]. As alternatives to MSCs, tolerogenic natural and artificial[173] antigen-presenting cells, human amniotic epithelial cells[174] and type 2 innate lymphoid cells[175], have also shown promising results. Other limitations include cell-sourcing challenges and regulatory considerations. The co-delivery of MSC-derived extracellular vesicles could circumvent these challenges.

Reducing immunogenicity of islet grafts

With advances in gene editing technologies, such as CRISPR–Cas9, it is now possible to genetically engineer islet allografts or stem cells before their differentiation into stem cell-derived islets to decrease their immunogenicity[176,177]. Relevant to this, in a clinical trial, recipients of donors positive for HLA-DQ8 had substantially better graft survival[178]. In another study, CRISPR–Cas9-engineered hypoimmune islet allografts remained functional and provided insulin independence in an

immunocompetent NHP model of diabetes, in the absence of immunosuppression[179]. Multiple approaches have been explored in preclinical rodent studies. These include knocking down expression of HLA class I and II while expressing HLA-E[180] in combination with overexpression of immunomodulatory signals, such as PDL1 and FasL (to induce T cell deletion and anergy)[181–183], CD47 ('don't eat me' signal)[184–186], HLA-G (tolerogenic placenta-mimicking signal for T cells)[187] and indoleamine dioxygenase[188]. As an alternative to genetic engineering, treatment of stem cell-derived islets with inflammatory cytokines to promote expression of immunomodulatory signals (PDL1)[189] and transplantation of islets from genetically engineered pigs[190,191] have also been tested in preclinical models. However, the effect of these genetic manipulations on core β cell function and glucose regulation remains to be elucidated. Moreover, given the ability of genetically modified cells to evade immune surveillance, on-demand safety switches are important[177].

Animal models

Animal models have a key role in T1DM research by facilitating the development and clinical translation of strategies for cell transplantation, cell delivery technologies and immunomodulatory interventions[192]. Immunocompromised models, often using mice, enable investigation of human islet and stem cell-derived islet engraftment without rejection[193]. Immunocompetent models, using rats, pigs and NHPs, can mimic different aspects of the immune response seen in humans, which are fundamental for evaluating inflammatory responses, FBR and immune protection strategies[194]. Humanized models incorporate human immune components to mimic the human immune system and, in some cases, T1DM pathophysiology[195]. Each model, discussed hereafter, serves a distinct purpose, such as elucidating engraftment mechanisms (mice), studying subdermal devices (pigs) and predicting outcomes in humans (NHPs). Their combined use is required to obtain a comprehensive and complementary assessment of therapeutic interventions for T1DM (Box 2).

Box 2 | Animal models for the development of β cell replacement products

Immunocompetent mouse or rat models
- Study islet metabolism and function
- Monitor rejection of islet allografts
- Model autoimmune diabetes
- Test immunotherapies
- Evaluate encapsulation strategies

Porcine models
- Evaluate islet transplant sites
- Study pancreatic development
- Test islet function in vivo
- Monitor rejection of islet allografts
- Source of islets for transplantation

Non-human primate models
- Biological complexity and physiological similarity to humans
- Test cross-reaction of biological agents and immunotherapies
- Enable clinical-trial-like study designs
- Monitor rejection of islet allografts

Humanized mouse models
- Model transplantation of human, porcine or stem cell-islet grafts
- Monitor rejection of islet allografts
- Potential to model human autoimmune diabetes
- Evaluate encapsulation strategies
- Test immunotherapies

Immunocompromised and immunocompetent rodent models

Inbred and transgenic mice are excellent and readily available model systems that faithfully recapitulate the in vivo homeostasis of islet grafts observed in larger mammals, including humans[196]. Combining immunodeficient *scid*, *Rag1*^{null} or *Rag2*^{null} mouse strains that bear mutations within the *Il2rg* chain eliminates mouse T cells, B cells and natural killer (NK) cells[197]. These severely immunodeficient mice support the transplant of human cadaveric islets, stem cell-derived islets and porcine islets, and are used to test the survival and functionality of the implanted populations[14,196]. Fully immunocompetent mouse models (such as C57BL/6 and BALB/C mice) have been used to evaluate the ability of genome engineering, drug and/or biomaterial or encapsulation approaches to confer protection from immune destruction[186,189,198,199]. NOD mice are fully immunocompetent and develop spontaneous diabetes, providing a platform to test autoimmune interactions and therapeutic interventions[200]. However, investigators must consider various inherent species differences between NOD mice and humans with T1DM (including incidence rates, peri-insulitis, immune cell infiltration and autoantigens); extrapolating preclinical findings to the clinical setting without proper validation should be avoided[200]. To study the T cell epitope repertoire in humans, researchers developed HLA-restricted mouse T cells[201,202]. HLA-matched islet grafts are attacked by autoimmune T cells, providing a powerful model to study autoimmunity[203]. Indeed, although some studies have found that overexpression of the immune checkpoint inhibitor PDL1 can provide effective protection from xenogeneic immune destruction[190], this strategy is not successful using this humanized mouse model[182].

Rat models of T1DM provide versatility through spontaneous, chemically induced and genetically manipulated variants. Chemically induced models (streptozotocin and alloxan) are cost-effective and reliable, yield diverse outcomes based on dosage[204], and can capture some T1DM complications, including neuropathy[205]. Sprague–Dawley rats with cyclophosphamide-induced diabetes are employed for studying diabetes pathogenesis[206]. The BioBreeding (BB) rat model, LEW.1AR1/-iddm, and Buffalo rats closely emulate T1DM autoimmune models, although BB rats exhibit lymphopenia not found in humans[207]. BB rats feature pancreatic insulitis and express the RT1 B/Du class II allele. Induction methods involve T_{reg} cell depletion, Toll-like receptor ligation and viral infections[208]. The LEW.1WE1 rat model also develops spontaneous diabetes, while the modified LEW.1AR1/Ztm0Dock7m^{+} rat and Worcester Founder rats have been used to study T1DM susceptibility genes[209]. For studies on subcutaneous devices, rat models are often preferred over mouse models because of the thick subcutaneous fat layer that more closely resembles the characteristics of human skin. Moreover, these models are useful in early valuation of encapsulation devices given their larger size and longer diffusion distances.

Humanized mouse models

Humanized mouse models are being used to study β cell replacement therapies and the immunogenicity of β cells[210,211], including β cell

function and safety, immune interactions, therapeutic interventions to prevent rejection, encapsulation strategies and genetic modifications of β cells to enhance cell function and survival[193]. Humanized mouse models are based on immunodeficient mouse strains discussed above. An advantage of humanized models is their ability to support engraftment of functional human immune systems by injection of human peripheral blood mononuclear cells (PBMCs), haematopoietic stem cells or thymus and/or liver tissues[212]. The immunogenicity of the transplanted islets can be directly assessed in humanized mice engrafted with human immune systems that are either mismatched with the islets to study alloreactivity[213] or from an autologous donor to study autoreactivity[214]. In the context of alloreactivity, humanized mice have been used to test immunotherapeutics, chimeric antigen receptor T_{reg} cells and effector cells, encapsulation strategies and genetic modification to prevent rejection[189,197,215–217]. The study of autoimmune responses is a challenge in humanized mice, but implantation of human iPSC-derived islets and engraftment of PBMCs from the autologous donor might enable the study of autoimmune T1DM[186]. In addition, the expression of autoreactive T cell receptors in human T cells is being used as a surrogate to study autoimmunity against implanted human islets[218]. Limitations of humanized mouse models include strain selection, development and maintenance of specific immune cells in vivo, graft-versus-host disease (GVHD), limited experimental timeframes, technical aspects for cell and tissue implantation, and overall cost.

Porcine models

Regulatory agencies are likely to require large, non-rodent animal model studies before evaluating islet transplantation in humans. Humans and pigs are omnivorous mammals with similar physiology and metabolic diseases, including type 2 diabetes mellitus, obesity, insulinopathy and β cell stress[219]. The immune system of pigs also has many similarities to that of humans, including immune cell subsets, trafficking and organization of lymphoid tissues[220]. Porcine metabolism is also more closely related to human metabolism than is NHP metabolism, making pigs a highly human-relevant model for islet transplantation studies[221]. Moreover, pig skin resembles human skin more closely than that of rodents or NHPs, making porcine models more appropriate for testing subcutaneous cell transplantation. Feasibility studies support the use of a porcine islet transplant model in preclinical studies. A porcine model of diabetes[222] has been successfully used to assess the feasibility of neonatal porcine islet grafts to reverse hyperglycaemia in allogeneic recipients[223]. Non-diabetic pigs have been used to assess the survival of allogeneic porcine islets transplanted into the subcutaneous space created using polymer scaffolds[224,225]. Pigs have also been used as a model for islet cell development that is relevant to human pancreatic development[226].

Non-human primate models

Given the genetic, physiological and behavioural parallels between NHPs and humans, NHPs are often the preferred translational model for exploring intricate biological systems and disease processes[227]. This is especially true in comprehending the immune response that mirrors the complexities of human immunity and mimics the physiology of transplantation[228]. NHP models have had a crucial role in T1DM research[229], particularly in islet transplantation, making substantial contributions to successful strategies aimed at preventing organ rejection by evading immune surveillance or establishing immune tolerance. The immune system of NHPs is typically required to bridge promising studies in rodents to applications in humans[230,231]. This is evidenced by the successful translation of new immunosuppressive drugs, such as alemtuzumab and belatacept, and regimens that foster immune tolerance from NHP studies to clinical applications[227].

NHPs are particularly pertinent in addressing the ongoing shortage of organ donors and exploring innovative cell sources, such as grafts from pigs or the utilization of stem cells. The pig-to-NHP xenograft model has provided researchers with a deeper understanding of the fundamental biological differences between pigs and primates that influence graft rejection, emphasizing the insufficiency of conventional immunosuppression in the xenograft context[232,233] and revealing targets necessary for success (such as blockade of the CD40–CD154 co-stimulatory pathway)[234]. Similarly, NHP modelling has also facilitated the identification of targets for gene editing as an intervention strategy[179,234]. NHP models can be leveraged to enable longitudinal studies of transplant function and risk, addressing persistent challenges in optimizing dosage, control over the immune response and delivery[9]. The striking similarity between NHPs and humans in terms of environmental exposures, size, drug effects and long lifespan places them among the most rigorous, informative and predictive non-clinical models for integrative studies in transplantation[235].

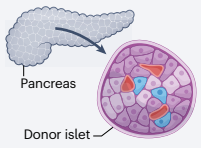
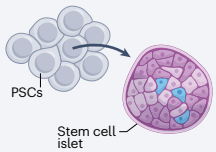
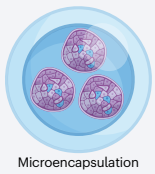
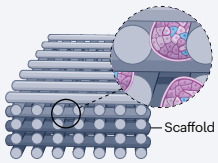
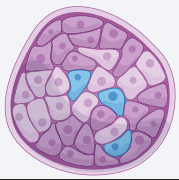
Guide to clinical translation

Consensus on preclinical testing

Harmonizing assessment protocols is essential for clear and direct comparison of results to accelerate the development of β cell replacement therapies. Stem cell-derived β cell preparations should be carefully characterized by assessing cell composition and functional properties and benchmarking against the latest state-of-the-art protocols. Currently, these protocols, which entail differentiation over 24 days and maturation over 6 weeks in culture, generate cell populations consisting of ~40% monohormonal insulin-positive cells, 40–50% monohormonal glucagon-positive cells, 5% somatostatin-positive cells and 5–15% enterochromaffin cells[14,15,23,32,117,236–238]. Functional assessments should include insulin content as well as glucose-stimulated insulin secretion assays to determine responses to changes in environmental glucose levels and exposure to other secretagogues (such as exendin-4, tolbutamide and KCl). Dynamic perfusion assays are preferred, as these provide insights into the kinetics of insulin secretion. The research community should be consistent in secretagogue concentrations used during testing to enable clear and direct comparisons, with 3 mM and 16 mM glucose for low-glucose and high-glucose buffers, respectively, as is frequently observed in the literature. Additionally, stem cell-derived β cell preparations should be evaluated for measurements of ion channel conductance, cytoplasmic calcium ions, cAMP concentrations and exocytosis to determine whether cells have acquired the machinery required for glucose-responsive insulin secretion.

Rodent studies are the first step in testing and validating various approaches in vivo. For cell delivery devices and other biomaterial-based strategies for cell survival, vascularization and engraftment, biocompatibility should be tested alone (without cells), followed by histological evaluation of tissue responses. Next, syngeneic immunocompetent rodent models should be used to analyse innate immune responses and engraftment in cell transplantation without the confounding aspects of adaptive immune rejection. Here, key analyses include assessment of fibrosis via Masson's trichrome staining, diabetes reversal timing and rate, and necessary cell dosages to achieve normoglycaemia. Intraperitoneal glucose tolerance tests (IPGTT) and glucose area under the curve (AUC) analysis should be

Table 1 | Proposed iterative developments of β cell replacement products

	Current islet transplantation	First-generation naked β cell therapy	Second-generation β cell therapy: encapsulation	Second-generation β cell therapy: other protections	Future β cell therapy
Product schematics					
Cell source	Donor islet transplant	Renewable source	Renewable source	Renewable source	Renewable source
Immune suppression	Broad suppression	Broad suppression	No broad suppression	Reduced/no broad suppression	No broad suppression
Glycaemic control	Improved (HbA_{1c}, hypos, TIR)	Improved (HbA_{1c}, hypos, TIR)	Improved (HbA_{1c}, hypos, TIR)	Improved (HbA_{1c}, hypos, TIR)	Physiological glucose regulation restored
Daily insulin requirement	50–100% reduction	50–100% reduction	70–100% reduction	70–100% reduction	100% reduction (insulin independence)
Duration of efficacy	Variable	6–24 months	6–24 months	6–24 months	>24 months

hypos, hypoglycaemic events; PSC, pluripotent stem cell; TIR, time in range.

performed. Detailed imaging to quantify functional vascularization, vessel density and branching, and integration with host tissues are also crucial.

To evaluate immune protection or immune tolerance, short-term (30–45 days) syngeneic rodent models should confirm no negative effects on engraftment and function (no-harm studies), followed by efficacy assessment in discordant allogeneic models over a minimum of 100 days. Key outcomes remain diabetes reversal and cell dose efficacy, confirmed using IPGTT and glucose AUC analysis. Further, mechanistic studies characterizing immune response dynamics, donor tissue sensitization, third-party immune challenge response, and evaluation of local and systemic effects are vital. To increase robustness and evaluate immune protection approaches in the context of autoimmunity, syngeneic transplants in the NOD model can be used. For both autoimmunity and alloimmunity, allogeneic transplants in spontaneously diabetic NOD mice can be used. Humanized mouse models can also be used when testing immune protection strategies incorporating human cells but, given their limitations due to lack of certain immune cell compartments (that is, NK cells) and GVHD, these studies should be complemented by other in vitro testing.

As various cell delivery and immune protection strategies prove successful in rodents, the assessments described above should also be performed in larger animals, such as pigs or NHPs. to enable translation towards the clinic. Immune protection strategies should be assessed in these models for at least 6 months. Testing human cells in immunocompetent animal models might not be necessary as it introduces confounding factors related to xenogeneic immune responses. In fact, studies in immunocompromised animals demonstrating safety and efficacy of the complete combination product using human cells, along with complementary data in immunocompetent models using allogeneic cells, may suffice. Finally, developers should engage with regulators early to discuss their development plans and obtain feedback.

Roadmap for β cell replacement products

To create a complete β cell replacement product, three main elements, discussed extensively above, must converge: one, a renewable source of insulin-producing cells; two, a cell delivery strategy that ensures survival, engraftment and function of these cells; and three, an approach to prevent long-term immune rejection of the transplant. Achieving all of these concurrently in a first-generation product poses considerable technical and regulatory challenges. Despite these hurdles, realistic near-term and long-term projections of likely β cell replacement products can be made to guide their development as the field pushes towards an aspirational product. To this end, Breakthrough T1D (formerly JDRF), the world's largest non-profit funder of T1DM research and a key advocacy group for the T1DM community, has worked closely with key opinion leaders to outline a developmental roadmap (Table 1). This plan envisions the evolution of a series of products over a multistage development pathway and patient segments. Each product is expected to offer the optimal balance of benefits to risks for a targeted population and to present viable market opportunities for cell therapy among the individuals of that population[239]. In this phased approach, each product is anticipated to add complexity and surpass the previous version by improving glycaemic control and immune protection, thus reducing the need for systemic immunosuppression, extending graft longevity and expanding the population that is eligible to receive these therapies.

Cadaveric islet transplantation, approved in the USA, Europe and Australia, relies on donor islets and chronic systemic immunosuppression to protect islets from immune rejection, limiting the patient populations that could benefit from and have access to this therapy. Vertex's VX-880 clinical trial has shown encouraging results[17] and represents a first-generation β cell replacement product in which cadaveric islets are substituted by human stem cell-derived islets, with immune protection still achieved via chronic systemic immunosuppression. Approaches to provide immune protection, including encapsulation or immunomodulation, will be introduced to eliminate the need for systemic immunosuppression to advance towards second-generation products. These second-generation products entailing cell encapsulation must overcome challenges with FBR and limited mass transfer. Advances are focusing on integration with genetically modified immune-evasive cells. As the field progresses in the development of immune protection strategies and immune evasive cell sources, alternative transplantation

sites for insulin-producing cells, such as subcutaneous or omental implantation, must be explored to improve cell survival and function and mitigate risk by enabling facile monitoring and graft retrieval in case of adverse events in second-generation and third-generation (aspirational) products.

Ultimately, the goal is to make a reproducible and safe product capable of restoring physiological blood glucose control and insulin independence for a prolonged period (10+ years) without the use of systemic immunosuppression. As product developers work towards these challenging goals, it will be critical to carefully define the therapeutic concepts and target product profiles[240]. Target product profiles are meant to delineate the intended use and product indication, the target patient population, desired product attributes, anticipated outcomes and efficacy measures, and potential risks and side effects. These will be crucial for assessing market potential, guiding development and framing submissions for regulatory approval.

Future perspectives and challenges

The past decade has witnessed substantial progress in cell transplantation for T1DM management[5], with stem cell-derived islets offering hope for a clinically viable breakthrough. Several consortia including the DRIVE Consortium[241], the Beta Cell Therapy Consortium[242], and the Breakthrough T1D Beta Cell Replacement Consortium[243] have been established across Europe and North America to gather experts from various relevant fields from both industry and academia and to foster a comprehensive understanding of the complex challenges involved. These consortia enable the exchange of information, reagents and methods as well as the establishment of critical collaborations between teams with the complementary expertise needed to accelerate progress. Moreover, these consortia enable the pairing and integration of complementary technologies early in development to explore potential synergies that might lead to products that can deliver superior outcomes. Through this collaborative approach, researchers can overcome barriers more efficiently.

Transplantation of alginate microencapsulated cadaveric islets is probably at the forefront for clinical testing, not because of superiority, but due to their extensive research foundation and regulatory alignment, which could benefit from the precedent set by the approval of donislecel[244]. Although this method could be clinically accepted, it does not resolve the challenge of donor islet shortage or limited efficacy after prolonged periods. Renewable β cell sources are transitioning into advanced clinical evaluation. Novel technologies, such as CRISPR–Cas9 could further tailor these renewable cell sources to thrive in challenging anatomical sites, increasing their robustness.

In parallel, emerging approaches are refining cell delivery strategies, aiming at modulating anti-β cell immune responses while enhancing resistance to hypoxic conditions at implantation sites. These innovations are directed towards incorporating hypoimmunogenic cells within biomaterials, devices or scaffolds, coupled with the delivery of localized immunosuppressive agents. Additionally, the demonstrated ability to generate and sustain a patent vascular network in tissues, achieving enhanced partial oxygen pressure with respect to the native tissue microenvironment, has highlighted the relevance of subcutaneous cell deployment. As such, subcutaneous islet transplantation, which promises minimally invasive and reversible treatment options, is likely to remain a focal point in the development of novel encapsulation devices. In case of malfunction, infections or failure of the graft, subcutaneous systems can be safely removed and replaced. Achieving normoglycaemia through subcutaneous cell transplantation without systemic immunosuppression, multiple daily exogenous insulin injections, and risk of hyperglycaemic or hypoglycaemic events would represent an enormous improvement in the quality of life for individuals with T1DM. Patients would be relieved of continuous self-monitoring of glucose levels and the stress of insulin administration.

Complex biomimetic approaches are being actively investigated as the next generation of cell therapeutics for T1DM. Cell therapy strategies aim to closely replicate and restore the natural functions of dysfunctional tissue or cells, which ideally involves engrafting therapeutic cells within a microenvironment that not only mimics the healthy native organ but is also situated in the organ's natural anatomical location. For T1DM, the optimal scenario would be to transplant islets within their niche in the pancreas, but owing to access and retrievability concerns, alternative sites have been targeted. Although the pursuit of such biomimetic approaches is scientifically and theoretically appealing, the search for a widely applicable clinical solution must find a compromise between the ideal and the practical. Achieving a balance that accounts for technological feasibility, surgical viability, efficacy and off-target effects, patient comfort and acceptance, the regulatory pathway, health-care economics and economic sustainability, requires a carefully engineered approach. To this end, reaching a consensus, harmonizing methods, identifying success criteria and leveraging animal models to evaluate different technologies under development will significantly accelerate progress.

Conclusions

Substantial advances have been made in cell transplantation for the treatment of T1DM since the Edmonton protocol, including the identification of potential renewable cell sources and safer, more effective immune-protective delivery methods and sites. Ideally, insights gained from each of the developments and technologies discussed in this Review can be appropriately and optimally integrated to yield a final cell product that is universally applicable to a large population of individuals with T1DM. Furthermore, innovations in this front can be truly transformative if they can also address the challenge of universal accessibility in the context of socioeconomic and geographic disparities.

Published online: 3 September 2024

References

1. International Diabetes Federation. *IDF Diabetes Atlas Reports: Type 1 Diabetes Estimates in Children and Adults* (International Diabetes Federation, 2022).
2. US Centers for Disease Control and Prevention. *National Diabetes Statistics Report* (CDC, 2024).
3. Tonnies, T. et al. Projections of type 1 and type 2 diabetes burden in the U.S. population aged <20 years through 2060: the SEARCH for diabetes in youth study. *Diabetes Care* **46**, 313–320 (2023).
4. Syed, F. Z. Type 1 diabetes mellitus. *Ann. Intern. Med.* **175**, ITC33–ITC48 (2022).
5. Ebekozien, O. et al. Longitudinal trends in glycemic outcomes and technology use for over 48,000 people with type 1 diabetes (2016-2022) from the T1D exchange quality improvement collaborative. *Diabetes Technol. Ther.* **25**, 765–773 (2023).
6. Wilson, L. M., Jacobs, P. G., Riddell, M. C., Zaharieva, D. P. & Castle, J. R. Opportunities and challenges in closed-loop systems in type 1 diabetes. *Lancet Diabetes Endocrinol.* **10**, 6–8 (2022).
7. Marfil-Garza, B. A. et al. Pancreatic islet transplantation in type 1 diabetes: 20-year experience from a single-centre cohort in Canada. *Lancet Diabetes Endocrinol.* **10**, 519–532 (2022).
8. Vantyghem, M. C., de Koning, E. J. P., Pattou, F. & Rickels, M. R. Advances in beta-cell replacement therapy for the treatment of type 1 diabetes. *Lancet* **394**, 1274–1285 (2019).
9. Butler, P. C. & Gale, E. A. Reversing type 1 diabetes with stem cell-derived islets: a step closer to the dream? *J. Clin. Invest.* **132**, e158305 (2022).
10. Paez-Mayorga, J. et al. Emerging strategies for beta cell transplantation to treat diabetes. *Trends Pharmacol. Sci.* **43**, 221–233 (2022).
11. Roberts, M. B. & Fishman, J. A. Immunosuppressive agents and infectious risk in transplantation: managing the "net state of Immunosuppression". *Clin. Infect. Dis.* **73**, e1302–e1317 (2021).

12. Wehner, M. R. et al. Risks of multiple skin cancers in organ transplant recipients: a cohort study in 2 administrative data sets. *JAMA Dermatol.* **157**, 1447–1455 (2021).
13. Shapiro, A. M. J. & Verhoeff, K. A spectacular year for islet and stem cell transplantation. *Nat. Rev. Endocrinol.* **19**, 68–69 (2023).
14. Pagliuca, F. W. et al. Generation of functional human pancreatic beta cells in vitro. *Cell* **159**, 428–439 (2014).
15. Rezania, A. et al. Reversal of diabetes with insulin-producing cells derived in vitro from human pluripotent stem cells. *Nat. Biotechnol.* **32**, 1121–1133 (2014).
16. US National Library of Medicine. *CinicalTrials.gov* www.clinicaltrials.gov/study/NCT04786262 (2024).
17. Businesswire. Vertex presents positive, updated VX-880 results from ongoing phase 1/2 study in type 1 diabetes at the European Association for the Study of Diabetes 59th Annual Meeting. *Businesswire* www.businesswire.com/news/home/20231003786678/en/Vertex-Presents-Positive-Updated-VX-880-Results-From-Ongoing-Phase-12-Study-in-Type-1-Diabetes-at-the-European-Association-for-the-Study-of-Diabetes-59th-Annual-Meeting (2023).
18. Vertex Pharmaceuticals. Vertex provides pipeline and business updates in advance of upcoming investor meetings. *Vertex* news.vrtx.com/news-releases/news-release-details/vertex-provides-pipeline-and-business-updates-advance-upcoming (2024).
19. Hogrebe, N. J., Ishahak, M. & Millman, J. R. Developments in stem cell-derived islet replacement therapy for treating type 1 diabetes. *Cell Stem Cell* **30**, 530–548 (2023).
20. Augsornworawat, P. et al. Single-nucleus multi-omics of human stem cell-derived islets identifies deficiencies in lineage specification. *Nat. Cell Biol.* **25**, 904–916 (2023).
21. Zhu, H. et al. Understanding cell fate acquisition in stem-cell-derived pancreatic islets using single-cell multiome-inferred regulomes. *Dev. Cell* **58**, 727–743.e11 (2023).
22. Veres, A. et al. Charting cellular identity during human in vitro β-cell differentiation. *Nature* **569**, 368–373 (2019).
23. Balboa, D. et al. Functional, metabolic and transcriptional maturation of human pancreatic islets derived from stem cells. *Nat. Biotechnol.* **40**, 1042–1055 (2022).
24. Augsornworawat, P., Velazco-Cruz, L., Song, J. & Millman, J. R. A hydrogel platform for in vitro three dimensional assembly of human stem cell-derived islet cells and endothelial cells. *Acta Biomater.* **97**, 272–280 (2019).
25. Mahaddalkar, P. U. et al. Generation of pancreatic β cells from CD177^{+} anterior definitive endoderm. *Nat. Biotechnol.* **38**, 1061–1072 (2020).
26. Aghazadeh, Y. et al. GP2-enriched pancreatic progenitors give rise to functional beta cells in vivo and eliminate the risk of teratoma formation. *Stem Cell Rep.* **17**, 964–978 (2022).
27. Maxwell, K. G., Kim, M. H., Gale, S. E. & Millman, J. R. Differential function and maturation of human stem cell-derived islets after transplantation. *Stem Cell Transl. Med.* **11**, 322–331 (2022).
28. Ryan, E. A. et al. Successful islet transplantation: continued insulin reserve provides long-term glycemic control. *Diabetes* **51**, 2148–2157 (2002).
29. Markmann, J. F. et al. Insulin independence following isolated islet transplantation and single islet infusions. *Ann. Surg.* **237**, 741–749 (2003).
30. Niebruegge, S. et al. Generation of human embryonic stem cell-derived mesoderm and cardiac cells using size-specified aggregates in an oxygen-controlled bioreactor. *Biotechnol. Bioeng.* **102**, 493–507 (2009).
31. Ismadi, M. Z. et al. Flow characterization of a spinner flask for induced pluripotent stem cell culture application. *PLoS ONE* **9**, e106493 (2014).
32. Hogrebe, N. J., Augsornworawat, P., Maxwell, K. G., Velazco-Cruz, L. & Millman, J. R. Targeting the cytoskeleton to direct pancreatic differentiation of human pluripotent stem cells. *Nat. Biotechnol.* **38**, 460–470 (2020).
33. Paull, D. et al. Automated, high-throughput derivation, characterization and differentiation of induced pluripotent stem cells. *Nat. Methods* **12**, 885–892 (2015).
34. Millman, J. R., Tan, J. H. & Colton, C. K. Mouse pluripotent stem cell differentiation under physiological oxygen reduces residual teratomas. *Cell Mol. Bioeng.* **14**, 555–567 (2021).
35. Fong, C. Y., Gauthaman, K. & Bongso, A. Teratomas from pluripotent stem cells: a clinical hurdle. *J. Cell Biochem.* **111**, 769–781 (2010).
36. Laurent, L. C. et al. Dynamic changes in the copy number of pluripotency and cell proliferation genes in human ESCs and iPSCs during reprogramming and time in culture. *Cell Stem Cell* **8**, 106–118 (2011).
37. Garber, K. RIKEN suspends first clinical trial involving induced pluripotent stem cells. *Nat. Biotechnol.* **33**, 890–891 (2015).
38. Taapken, S. M. et al. Karotypic abnormalities in human induced pluripotent stem cells and embryonic stem cells. *Nat. Biotechnol.* **29**, 313–314 (2011).
39. Merkle, F. T. et al. Human pluripotent stem cells recurrently acquire and expand dominant negative P53 mutations. *Nature* **545**, 229–233 (2017).
40. Rouhani, F. J. et al. Substantial somatic genomic variation and selection for BCOR mutations in human induced pluripotent stem cells. *Nat. Genet.* **54**, 1406–1416 (2022).
41. Roschke, A. V. & Kirsch, I. R. Targeting karyotypic complexity and chromosomal instability of cancer cells. *Curr. Drug. Targets* **11**, 1341–1350 (2010).
42. Muller, P. A. & Vousden, K. H. p53 mutations in cancer. *Nat. Cell Biol.* **15**, 2–8 (2013).
43. Astolfi, A. et al. BCOR involvement in cancer. *Epigenomics* **11**, 835–855 (2019).
44. Hirai, T., Yasuda, S., Umezawa, A. & Sato, Y. Country-specific regulation and international standardization of cell-based therapeutic products derived from pluripotent stem cells. *Stem Cell Rep.* **18**, 1573–1591 (2023).
45. International Society for Stem Cell Research. *Guidelines for Stem Cell Research and Clinical Translation* (International Society for Stem Cell Research, 2022).
46. US Food and Drug Administration. *Regulatory Considerations for Human Cells, Tissues, and Cellular and Tissue-based Products: Minimal Manipulation and Homologous Use* (FDA, 2020).
47. Yamamoto, T. et al. Quality control for clinical islet transplantation: organ procurement and preservation, the islet processing facility, isolation, and potency tests. *J. Hepatobiliary Pancreat. Surg.* **16**, 131–136 (2009).
48. Ricordi, C. et al. Improved human islet isolation outcome from marginal donors following addition of oxygenated perfluorocarbon to the cold-storage solution. *Transplantation* **75**, 1524–1527 (2003).
49. Walker, S., Appari, M. & Forbes, S. Considerations and challenges of islet transplantation and future therapies on the horizon. *Am. J. Physiol. Endocrinol. Metab.* **322**, E109–E117 (2022).
50. Lee, K. Y. & Mooney, D. J. Alginate: properties and biomedical applications. *Prog. Polym. Sci.* **37**, 106–126 (2012).
51. Hu, S. et al. Toll-like receptor 2-modulating pectin-polymers in alginate-based microcapsules attenuate immune responses and support islet-xenograft survival. *Biomaterials* **266**, 120460 (2021).
52. Bennet, W., Groth, C. G., Larsson, R., Nilsson, B. & Korsgren, O. Isolated human islets trigger an instant blood mediated inflammatory reaction: implications for intraportal islet transplantation as a treatment for patients with type 1 diabetes. *Ups. J. Med. Sci.* **105**, 125–133 (2000).
53. van der Windt, D. J., Bottino, R., Casu, A., Campanile, N. & Cooper, D. K. Rapid loss of intraportally transplanted islets: an overview of pathophysiology and preventive strategies. *Xenotransplantation* **14**, 288–297 (2007).
54. Matsumoto, S. et al. Clinical porcine islet xenotransplantation under comprehensive regulation. *Transpl. Proc.* **46**, 1992–1995 (2014).
55. Huang, L. et al. Regulation of blood glucose using islets encapsulated in a melanin-modified immune-shielding hydrogel. *ACS Appl. Mater. Interfaces* **13**, 12877–12887 (2021).
56. Schaschkow, A. et al. Glycaemic control in diabetic rats treated with islet transplantation using plasma combined with hydroxypropylmethyl cellulose hydrogel. *Acta Biomater.* **102**, 259–272 (2020).
57. Kuwabara, R. et al. Extracellular matrix inclusion in immunoisolating alginate-based microcapsules promotes longevity, reduces fibrosis, and supports function of islet allografts in vivo. *Acta Biomater.* **158**, 151–162 (2023).
58. Elliott, R. B. et al. Live encapsulated porcine islets from a type 1 diabetic patient 9.5 yr after xenotransplantation. *Xenotransplantation* **14**, 157–161 (2007).
59. Harrington, S., Williams, J., Rawal, S., Ramachandran, K. & Stehno-Bittel, L. Hyaluronic acid/collagen hydrogel as an alternative to alginate for long-term immunoprotected islet transplantation. *Tissue Eng. Part. A* **23**, 1088–1099 (2017).
60. Medina, J. D. et al. Functionalization of alginate with extracellular matrix peptides enhances viability and function of encapsulated porcine islets. *Adv. Healthc. Mater.* **9**, e2000102 (2020).
61. Alagpulinsa, D. A. et al. Alginate-microencapsulation of human stem cell-derived β cells with CXCL12 prolongs their survival and function in immunocompetent mice without systemic immunosuppression. *Am. J. Transpl.* **19**, 1930–1940 (2019).
62. Chen, T. et al. Alginate encapsulant incorporating CXCL12 supports long-term allo- and xenoislet transplantation without systemic immune suppression. *Am. J. Transpl.* **15**, 618–627 (2015).
63. Sremac, M. et al. Preliminary studies of the impact of CXCL12 on the foreign body reaction to pancreatic islets microencapsulated in alginate in nonhuman primates. *Transpl. Direct* **5**, e447 (2019).
64. Zhang, Q. et al. Islet encapsulation: new developments for the treatment of type 1 diabetes. *Front. Immunol.* **13**, 869984 (2022).
65. Lew, B., Kim, I. Y., Choi, H. & Kim, K. K. Sustained exenatide delivery via intracapsular microspheres for improved survival and function of microencapsulated porcine islets. *Drug. Deliv. Transl. Res.* **8**, 857–862 (2018).
66. Vaithilingam, V. et al. Co-encapsulation and co-transplantation of mesenchymal stem cells reduces pericapsular fibrosis and improves encapsulated islet survival and function when allografted. *Sci. Rep.* **7**, 10059 (2017).
67. Cheng, Y., Liu, Y. F., Zhang, J. L., Li, T. M. & Zhao, N. Elevation of vascular endothelial growth factor production and its effect on revascularization and function of graft islets in diabetic rats. *World J. Gastroenterol.* **13**, 2862–2866, (2007).
68. Tomei, A. A. et al. Device design and materials optimization of conformal coating for islets of Langerhans. *Proc. Natl Acad. Sci. USA* **111**, 10514–10519 (2014).
69. Manzoli, V. et al. Immunoisolation of murine islet allografts in vascularized sites through conformal coating with polyethylene glycol. *Am. J. Transpl.* **18**, 590–603 (2018).
70. Stock, A. A. et al. Conformal coating of stem cell-derived islets for β cell replacement in type 1 diabetes. *Stem Cell Rep.* **14**, 91–104 (2020).
71. Desai, T. A. & Tang, Q. Islet encapsulation therapy – racing towards the finish line? *Nat. Rev. Endocrinol.* **14**, 630–632 (2018).
72. Scharp, D. W. & Marchetti, P. Encapsulated islets for diabetes therapy: history, current progress, and critical issues requiring solution. *Adv. Drug. Deliv. Rev.* **67-68**, 35–73 (2014).
73. Scharp, D. W. et al. Protection of encapsulated human islets implanted without immunosuppression in patients with type I or type II diabetes and in nondiabetic control subjects. *Diabetes* **43**, 1167–1170 (1994).
74. Chang, R. et al. Nanoporous immunoprotective device for stem-cell-derived β-cell replacement therapy. *ACS Nano* **11**, 7747–7757 (2017).
75. Ludwig, B. et al. Transplantation of human islets without immunosuppression. *Proc. Natl Acad. Sci. USA* **110**, 19054–19058 (2013).

76. An, D. et al. Designing a retrievable and scalable cell encapsulation device for potential treatment of type 1 diabetes. *Proc. Natl Acad. Sci. USA* **115**, E263–E272 (2018).
77. Hwang, D. G. et al. A 3D bioprinted hybrid encapsulation system for delivery of human pluripotent stem cell-derived pancreatic islet-like aggregates. *Biofabrication* **14**, 014101 (2021).
78. Yang, K. et al. A therapeutic convection-enhanced macroencapsulation device for enhancing β cell viability and insulin secretion. *Proc. Natl Acad. Sci. USA* **118**, e2101258118 (2021).
79. Kharbikar, B. N., Chendke, G. S. & Desai, T. A. Modulating the foreign body response of implants for diabetes treatment. *Adv. Drug. Deliv. Rev.* **174**, 87–113 (2021).
80. Capuani, S., Malgir, G., Chua, C. Y. X. & Grattoni, A. Advanced strategies to thwart foreign body response to implantable devices. *Bioeng. Transl. Med.* **7**, e10300 (2022).
81. Avgoustiniatos, E. & Colton, C. in *Principles of Tissue Engineering* 1st edn (eds Lanza, R. P. et al.) 333–346 (Academic, 1997).
82. Colton, C. K. Oxygen supply to encapsulated therapeutic cells. *Adv. Drug. Deliv. Rev.* **67-68**, 93–110 (2014).
83. Papas, K. K., De Leon, H., Suszynski, T. M. & Johnson, R. C. Oxygenation strategies for encapsulated islet and beta cell transplants. *Adv. Drug. Deliv. Rev.* **139**, 139–156 (2019).
84. Wang, L. H. et al. An inverse-breathing encapsulation system for cell delivery. *Sci. Adv.* **7**, eabd5835 (2021).
85. Carlsson, P. O. et al. Transplantation of macroencapsulated human islets within the bioartificial pancreas βAir to patients with type 1 diabetes mellitus. *Am. J. Transpl.* **18**, 1735–1744 (2018).
86. Qin, T., Smink, A. M. & de Vos, P. Enhancing longevity of immunoisolated pancreatic islet grafts by modifying both the intracapsular and extracapsular environment. *Acta Biomater.* **167**, 38–53 (2023).
87. Valdes-Gonzalez, R. A. et al. Xenotransplantation of porcine neonatal islets of Langerhans and Sertoli cells: a 4-year study. *Eur. J. Endocrinol.* **153**, 419–427 (2005).
88. Birmingham, K. Skepticism surrounds diabetes xenograft experiment. *Nat. Med.* **8**, 1047 (2002).
89. Dufrane, D., Goebbels, R. M. & Gianello, P. Alginate macroencapsulation of pig islets allows correction of streptozotocin-induced diabetes in primates up to 6 months without immunosuppression. *Transplantation* **90**, 1054–1062 (2010).
90. Veriter, S. et al. Islets and mesenchymal stem cells co-encapsulation can improve subcutaneous bioartificial pancreas survival in diabetic primates. *Xenotransplantation* **18**, 276–276 (2011).
91. Chendke, G. S. et al. Supporting survival of transplanted stem-cell-derived insulin-producing cells in an encapsulation device augmented with controlled release of amino acids. *Adv. Biosyst.* **3**, 1900086 (2019).
92. Lottes, A. E. et al. Navigating the regulatory pathway for medical devices – a conversation with the FDA, clinicians, researchers, and industry experts. *J. Cardiovasc. Transl. Res.* **15**, 927–943 (2022).
93. Businesswire. Vertex announces positive day 90 data for the first patient in the phase 1/2 clinical trial dosed with VX-880, a novel investigational stem cell-derived therapy for the treatment of type 1 diabetes. *Businesswire* www.businesswire.com/news/home/20211018005226/en/ (2021).
94. US National Library of Medicine. *ClinicalTrials.gov* clinicaltrials.gov/study/NCT02239354 (2022).
95. US National Library of Medicine. *ClinicalTrials.gov* clinicaltrials.gov/study/NCT05791201 (2024).
96. Moruzzi, N., Leibiger, B., Barker, C. J., Leibiger, I. B. & Berggren, P. O. Novel aspects of intra-islet communication: primary cilia and filopodia. *Adv. Biol. Regul.* **87**, 100919 (2023).
97. Burganova, G., Bridges, C., Thorn, P. & Landsman, L. The role of vascular cells in pancreatic beta-cell function. *Front. Endocrinol.* **12**, 667170 (2021).
98. Almaca, J., Weitz, J., Rodriguez-Diaz, R., Pereira, E. & Caicedo, A. The pericyte of the pancreatic islet regulates capillary diameter and local blood flow. *Cell Metab.* **27**, 630–644.e4 (2018).
99. Bowers, D. T., Song, W., Wang, L. H. & Ma, M. Engineering the vasculature for islet transplantation. *Acta Biomater.* **95**, 131–151 (2019).
100. Yu, M. et al. Islet transplantation in the subcutaneous space achieves long-term euglycaemia in preclinical models of type 1 diabetes. *Nat. Metab.* **2**, 1013–1020 (2020).
101. Chua, C. Y. X. et al. Emerging immunomodulatory strategies for cell therapeutics. *Trends Biotechnol.* **41**, 358–373 (2023).
102. Forbes, S. et al. Human umbilical cord perivascular cells improve human pancreatic islet transplant function by increasing vascularization. *Sci. Transl. Med.* **12**, eaan5907 (2020).
103. Kinney, S. M., Ortaleza, K., Vlahos, A. E. & Sefton, M. V. Degradable methacrylic acid-based synthetic hydrogel for subcutaneous islet transplantation. *Biomaterials* **281**, 121342 (2022).
104. Weaver, J. D. et al. Vasculogenic hydrogel enhances islet survival, engraftment, and function in leading extrahepatic sites. *Sci. Adv.* **3**, e1700184 (2017).
105. Davalli, A. M. et al. A selective decrease in the beta cell mass of human islets transplanted into diabetic nude mice. *Transplantation* **59**, 817–820 (1995).
106. Aghazadeh, Y. et al. Microvessels support engraftment and functionality of human islets and hESC-derived pancreatic progenitors in diabetes models. *Cell Stem Cell* **28**, 1936–1949.e8 (2021).
107. Wrublewsky, S. et al. Co-transplantation of pancreatic islets and microvascular fragments effectively restores normoglycemia in diabetic mice. *NPJ Regen. Med.* **7**, 67 (2022).
108. Wang, D. et al. Hyaluronic acid methacrylate/pancreatic extracellular matrix as a potential 3D printing bioink for constructing islet organoids. *Acta Biomater.* **165**, 86–101 (2023).
109. Tremmel, D. M. et al. A human pancreatic ECM hydrogel optimized for 3-D modeling of the islet microenvironment. *Sci. Rep.* **12**, 7188 (2022).
110. Citro, A. et al. Directed self-assembly of a xenogeneic vascularized endocrine pancreas for type 1 diabetes. *Nat. Commun.* **14**, 878 (2023).
111. Guyette, J. P. et al. Perfusion decellularization of whole organs. *Nat. Protoc.* **9**, 1451–1468 (2014).
112. Citro, A. et al. Biofabrication of a vascularized islet organ for type 1 diabetes. *Biomaterials* **199**, 40–51 (2019).
113. Song, W. et al. Engineering transferrable microvascular meshes for subcutaneous islet transplantation. *Nat. Commun.* **10**, 4602 (2019).
114. Salg, G. A. et al. Toward 3D-bioprinting of an endocrine pancreas: a building-block concept for bioartificial insulin-secreting tissue. *J. Tissue Eng.* **13**, 20417314221091033 (2022).
115. Liang, J. P. et al. Engineering a macroporous oxygen-generating scaffold for enhancing islet cell transplantation within an extrahepatic site. *Acta Biomater.* **130**, 268–280 (2021).
116. Dolgin, E. Diabetes cell therapies take evasive action. *Nat. Biotechnol.* **40**, 291–295 (2022).
117. Velazco-Cruz, L. et al. Acquisition of dynamic function in human stem cell-derived β cells. *Stem Cell Rep.* **12**, 351–365 (2019).
118. Capuani, S., Campa-Carranza, J. N., Hernandez, N., Chua, C. Y. X. & Grattoni, A. Modeling of a bioengineered immunomodulating microenvironment for cell therapy. *Adv. Healthc. Mater.* https://doi.org/10.1002/adhm.202304003 (2024).
119. Jang, S. B. et al. DAMP-modulating nanoparticle for successful pancreatic islet and stem cell transplantation. *Biomaterials* **287**, 121679 (2022).
120. Shapiro, A. M. J. et al. Insulin expression and C-peptide in type 1 diabetes subjects implanted with stem cell-derived pancreatic endoderm cells in an encapsulation device. *Cell Rep. Med.* **2**, 100466 (2021).
121. Goswami, D. et al. Design considerations for macroencapsulation devices for stem cell derived islets for the treatment of type 1 diabetes. *Adv. Sci.* **8**, e2100820 (2021).
122. Pepper, A. R. et al. A prevascularized subcutaneous device-less site for islet and cellular transplantation. *Nat. Biotechnol.* **33**, 518–523 (2015).
123. Farina, M. et al. Transcutaneously refillable, 3D-printed biopolymeric encapsulation system for the transplantation of endocrine cells. *Biomaterials* **177**, 125–138 (2018).
124. Paez-Mayorga, J. et al. Implantable niche with local immunosuppression for islet allotransplantation achieves type 1 diabetes reversal in rats. *Nat. Commun.* **13**, 7951 (2022).
125. Pepper, A. R. et al. Transplantation of human pancreatic endoderm cells reverses diabetes post transplantation in a prevascularized subcutaneous site. *Stem Cell Rep.* **8**, 1689–1700 (2017).
126. Pepper, A. R. et al. Posttransplant characterization of long-term functional hESC-derived pancreatic endoderm grafts. *Diabetes* **68**, 953–962 (2019).
127. Sernova Corp. Sernova provides recap of 2023 accomplishments and anticipated 2024 milestones. *Sernova Corp* www.sernova.com/press/release/?id=388 (2024).
128. Paez-Mayorga, J. et al. Enhanced in vivo vascularization of 3D-printed cell encapsulation device using platelet-rich plasma and mesenchymal stem cells. *Adv. Healthc. Mater.* **9**, e2000670 (2020).
129. Kuppan, P. et al. Co-transplantation of human adipose-derived mesenchymal stem cells with neonatal porcine islets within a prevascularized subcutaneous space augments the xenograft function. *Xenotransplantation* **27**, e12581 (2020).
130. Wassmer, C. H. et al. Bio-engineering of pre-vascularized islet organoids for the treatment of type 1 diabetes. *Transpl. Int.* **35**, 10214 (2021).
131. Rickels, M. R. & Robertson, R. P. Pancreatic islet transplantation in humans: recent progress and future directions. *Endocr. Rev.* **40**, 631–668 (2019).
132. Chang, C. A. et al. Curative islet and hematopoietic cell transplantation in diabetic mice without toxic bone marrow conditioning. *Cell Rep.* **41**, 111615 (2022).
133. Eledon Pharmaceuticals. Eledon reports updated data from ongoing phase 1b trial evaluating tegoprubart for prevention of rejection in kidney transplantation. *Eledon Pharmaceuticals* ir.eledon.com/news-releases/news-release-details/eledon-reports-updated-data-ongoing-phase-1b-trial-evaluating (2023).
134. Wisel, S. A. et al. A multi-modal approach to islet and pancreas transplantation with calcineurin-sparing immunosuppression maintains long-term insulin independence in patients with type I diabetes. *Transpl. Int.* **36**, 11367 (2023).
135. Anwar, I. J. et al. The anti-CD40L monoclonal antibody AT-1501 promotes islet and kidney allograft survival and function in nonhuman primates. *Sci. Transl. Med.* **15**, eadf6376 (2023).
136. Lee, K., Nguyen, V., Lee, K. M., Kang, S. M. & Tang, Q. Attenuation of donor-reactive T cells allows effective control of allograft rejection using regulatory T cell therapy. *Am. J. Transpl.* **14**, 27–38 (2014).
137. Cabello-Kindelan, C. et al. Immunomodulation followed by antigen-specific T_{reg} infusion controls islet autoimmunity. *Diabetes* **69**, 215–227 (2020).
138. Yang, S. J. et al. Pancreatic islet-specific engineered T_{regs} exhibit robust antigen-specific and bystander immune suppression in type 1 diabetes models. *Sci. Transl. Med.* **14**, eabn1716 (2022).
139. Marshall, G. P. et al. Biomaterials-based nanoparticles conjugated to regulatory T cells provide a modular system for localized delivery of pharmacotherapeutic agents. *J. Biomed. Mater. Res. A* **111**, 185–197 (2023).

140. Sicard, A. et al. Donor-specific chimeric antigen receptor T_{regs} limit rejection in naive but not sensitized allograft recipients. *Am. J. Transpl.* **20**, 1562–1573 (2020).
141. Pierini, A. et al. T cells expressing chimeric antigen receptor promote immune tolerance. *JCI Insight* **2**, e92865 (2017).
142. Hu, M. et al. Low-dose interleukin-2 combined with rapamycin led to an expansion of $CD4^+CD25^+FOXP3^+$ regulatory T cells and prolonged human islet allograft survival in humanized mice. *Diabetes* **69**, 1735–1748 (2020).
143. Yu, A. et al. Selective IL-2 responsiveness of regulatory T cells through multiple intrinsic mechanisms supports the use of low-dose IL-2 therapy in type 1 diabetes. *Diabetes* **64**, 2172–2183 (2015).
144. Hering, B. J. et al. Factors associated with favourable 5 year outcomes in islet transplant alone recipients with type 1 diabetes complicated by severe hypoglycaemia in the Collaborative Islet Transplant Registry. *Diabetologia* **66**, 163–173 (2023).
145. Citro, A., Cantarelli, E., Pellegrini, S., Dugnani, E. & Piemonti, L. Anti-inflammatory strategies in intrahepatic islet transplantation: a comparative study in preclinical models. *Transplantation* **102**, 240–248 (2018).
146. Bachul, P. J. et al. Post-hoc analysis of a randomized, double blind, prospective study at the University of Chicago: additional standardizations of trial protocol are needed to evaluate the effect of a CXCR1/2 inhibitor in islet allotransplantation. *Cell Transpl.* **30**, 9636897211001774 (2021).
147. Ge, J. et al. Adjuvant conditioning induces an immunosuppressive milieu that delays alloislet rejection through the expansion of myeloid-derived suppressor cells. *Am. J. Transpl.* **23**, 935–945 (2023).
148. Velluto, D., Bojadzic, D., De Toni, T., Buchwald, P. & Tomei, A. A. Drug-integrating amphiphilic nanomaterial assemblies: 1. spatiotemporal control of cyclosporine delivery and activity using nanomicelles and nanofibrils. *J. Control. Rel.* **329**, 955–970 (2021).
149. Jamison, B. L. et al. Tolerogenic delivery of a hybrid insulin peptide markedly prolongs islet graft survival in the NOD mouse. *Diabetes* **71**, 483–496 (2022).
150. Kuppan, P. et al. Co-localized immune protection using dexamethasone-eluting micelles in a murine islet allograft model. *Am. J. Transpl.* **20**, 714–725 (2020).
151. Nguyen, T. T. et al. The impact of locally-delivered tacrolimus-releasing microspheres and polyethylene glycol-based islet surface modification on xenogeneic islet survival. *J. Control. Rel.* **336**, 274–284 (2021).
152. Nguyen, T. T. et al. Engineering "cell-particle hybrids" of pancreatic islets and bioadhesive FK506-loaded polymeric microspheres for local immunomodulation in xenogeneic islet transplantation. *Biomaterials* **221**, 119415 (2019).
153. Pathak, S. et al. Particulate-based single-dose local immunosuppressive regimen for inducing tolerogenic dendritic cells in xenogeneic islet transplantation. *Adv. Healthc. Mater.* **10**, e2001157 (2021).
154. Fan, Y., Zheng, X., Ali, Y., Berggren, P. O. & Loo, S. C. J. Local release of rapamycin by microparticles delays islet rejection within the anterior chamber of the eye. *Sci. Rep.* **9**, 3918 (2019).
155. Barra, J. M. et al. Localized cytotoxic T cell-associated antigen 4 and antioxidant islet encapsulation alters macrophage signaling and induces regulatory and anergic T cells to enhance allograft survival. *Am. J. Transpl.* **23**, 498–511 (2023).
156. Sremac, M. et al. Short-term function and immune-protection of microencapsulated adult porcine islets with alginate incorporating CXCL12 in healthy and diabetic non-human primates without systemic immune suppression: a pilot study. *Xenotransplantation* **30**, e12826 (2023).
157. Medina, J. D. et al. A hydrogel platform for co-delivery of immunomodulatory proteins for pancreatic islet allografts. *J. Biomed. Mater. Res. A* **110**, 1728–1737 (2022).
158. Li, Y. et al. Immunosuppressive PLGA TGF-β1 microparticles induce polyclonal and antigen-specific regulatory T cells for local immunomodulation of allogeneic islet transplants. *Front. Immunol.* **12**, 653088 (2021).
159. Skoumal, M. et al. Localized immune tolerance from FasL-functionalized PLG scaffolds. *Biomaterials* **192**, 271–281 (2019).
160. Coronel, M. M. et al. Immunotherapy via PD-L1-presenting biomaterials leads to long-term islet graft survival. *Sci. Adv.* **6**, eaba5573 (2020).
161. Polishevska, K. et al. Nanothin conformal coating with poly(N-vinylpyrrolidone) and tannic acid (PVPON/TA) preserves murine and human pancreatic islets function. *Pharmaceutics* **15**, 1137 (2023).
162. Gammon, J. M. et al. Engineering the lymph node environment promotes antigen-specific efficacy in type 1 diabetes and islet transplantation. *Nat. Commun.* **14**, 681 (2023).
163. Gooch, A. M., Chowdhury, S. S., Zhang, P. M., Hu, Z. M. & Westenfelder, C. Significant expansion of the donor pool achieved by utilizing islets of variable quality in the production of allogeneic "neo-islets", 3-D organoids of mesenchymal stromal and islet cells, a novel immune-isolating biotherapy for type I diabetes. *PLoS ONE* **18**, e0290460 (2023).
164. Navaei-Nigjeh, M. et al. Microfluidically fabricated fibers containing pancreatic islets and mesenchymal stromal cells improve longevity and sustained normoglycemia in diabetic rats. *Biofabrication* **15**, 015013 (2022).
165. Lachaud, C. C. et al. Umbilical cord mesenchymal stromal cells transplantation delays the onset of hyperglycemia in the RIP-B7.1 mouse model of experimental autoimmune diabetes through multiple immunosuppressive and anti-inflammatory responses. *Front. Cell Dev. Biol.* **11**, 1089817 (2023).
166. Kenyon, N. S. et al. Extended survival versus accelerated rejection of nonhuman primate islet allografts: effect of mesenchymal stem cell source and timing. *Am. J. Transpl.* **21**, 3524–3537 (2021).
167. Wei, L. et al. Protective effect of mesenchymal stem cells on isolated islets survival and against hypoxia associated with the HIF-1α/PFKFB3 pathway. *Cell Transpl.* **31**, 9636897211073127 (2022).
168. Wang, X. et al. Engineered immunomodulatory accessory cells improve experimental allogeneic islet transplantation without immunosuppression. *Sci. Adv.* **8**, eabn0071 (2022).
169. Nguyen, T. T. et al. Engineering of hybrid spheroids of mesenchymal stem cells and drug depots for immunomodulating effect in islet xenotransplantation. *Sci. Adv.* **8**, eabn8614 (2022).
170. Sohni, A. & Verfaillie, C. M. Mesenchymal stem cells migration homing and tracking. *Stem Cell Int.* **2013**, 130763 (2013).
171. Mohammadi, M. R. et al. Exosome loaded immunomodulatory biomaterials alleviate local immune response in immunocompetent diabetic mice post islet xenotransplantation. *Commun. Biol.* **4**, 685 (2021).
172. Wang, L. et al. Engineered cytokine-primed extracellular vesicles with high PD-L1 expression ameliorate type 1 diabetes. *Small* **19**, e2301019 (2023).
173. Neshat, S. Y. et al. Improvement of islet engrafts via T_{reg} induction using immunomodulating polymeric tolerogenic microparticles. *ACS Biomater. Sci. Eng.* **9**, 3522–3534 (2023).
174. Lebreton, F. et al. Mechanisms of immunomodulation and cytoprotection conferred to pancreatic islet by human amniotic epithelial cells. *Stem Cell Rev. Rep.* **18**, 346–359 (2022).
175. Huang, Q. et al. IL-10 producing type 2 innate lymphoid cells prolong islet allograft survival. *EMBO Mol. Med.* **12**, e12305 (2020).
176. Sackett, S. D. et al. Genetic engineering of immune evasive stem cell-derived islets. *Transpl. Int.* **35**, 10817 (2022).
177. Sackett, S. D., Rodriguez, A. & Odorico, J. S. The nexus of stem cell-derived beta-cells and genome engineering. *Rev. Diabet. Stud.* **14**, 39–50 (2017).
178. Forbes, S. et al. Islet transplantation outcomes in type 1 diabetes and transplantation of HLA-DQ8/DR4: results of a single-centre retrospective cohort in Canada. *EClinicalMedicine* **67**, 102333 (2024).
179. Hu, X. et al. Hypoimmune islets achieve insulin independence after allogeneic transplantation in a fully immunocompetent non-human primate. *Cell Stem Cell* **31**, 334–340.e5 (2024).
180. Parent, A. V. et al. Selective deletion of human leukocyte antigens protects stem cell-derived islets from immune rejection. *Cell Rep.* **36**, 109538 (2021).
181. Castro-Gutierrez, R., Alkanani, A., Mathews, C. E., Michels, A. & Russ, H. A. Protecting stem cell derived pancreatic beta-like cells from diabetogenic T cell recognition. *Front. Endocrinol.* **12**, 707881 (2021).
182. Santini-Gonzalez, J. et al. Human stem cell derived beta-like cells engineered to present PD-L1 improve transplant survival in NOD mice carrying human HLA class I. *Front. Endocrinol.* **13**, 989815 (2022).
183. Woodward, K. B. et al. Pancreatic islets engineered with a FasL protein induce systemic tolerance at the induction phase that evolves into long-term graft-localized immune privilege. *Am. J. Transpl.* **20**, 1285–1295 (2020).
184. Hu, X. et al. Hypoimmune induced pluripotent stem cells survive long term in fully immunocompetent, allogeneic rhesus macaques. *Nat. Biotechnol.* **42**, 413–423 (2024).
185. Shrestha, P. et al. Immune checkpoint CD47 molecule engineered islets mitigate instant blood-mediated inflammatory reaction and show improved engraftment following intraportal transplantation. *Am. J. Transpl.* **20**, 2703–2714 (2020).
186. Hu, X. et al. Human hypoimmune primary pancreatic islets avoid rejection and autoimmunity and alleviate diabetes in allogeneic humanized mice. *Sci. Transl. Med.* **15**, eadg5794 (2023).
187. Rao, J. S. et al. HLA-$G1^+$ expression in GGTA1KO pigs suppresses human and monkey anti-pig T, B and NK cell responses. *Front. Immunol.* **12**, 730545 (2021).
188. Paul, P. K. et al. Islet allografts expressing a PD-L1 and IDO fusion protein evade immune rejection and reverse preexisting diabetes in immunocompetent mice without systemic immunosuppression. *Am. J. Transpl.* **22**, 2571–2585 (2022).
189. Yoshihara, E. et al. Immune-evasive human islet-like organoids ameliorate diabetes. *Nature* **586**, 606–611 (2020).
190. Lei, Y. et al. Neonatal islets from human PD-L1 transgenic pigs reduce immune cell activation and cellular rejection in humanized nonobese diabetic-scid $IL2r\gamma^{null}$ mice. *Am. J. Transpl.* **24**, 20–29 (2024).
191. Carvalho Oliveira, M. et al. Generating low immunogenic pig pancreatic islet cell clusters for xenotransplantation. *J. Cell Mol. Med.* **24**, 5070–5081 (2020).
192. Lorberbaum, D. S., Sarbaugh, D. & Sussel, L. Leveraging the strengths of mice, human stem cells, and organoids to model pancreas development and diabetes. *Front. Endocrinol.* **13**, 1042611 (2022).
193. Khosravi-Maharlooei, M. et al. Modeling human T1D-associated autoimmune processes. *Mol. Metab.* **56**, 101417 (2022).
194. Kottaisamy, C. P. D., Raj, D. S., Prasanth Kumar, V. & Sankaran, U. Experimental animal models for diabetes and its related complications – a review. *Lab. Anim. Res.* **37**, 23 (2021).
195. Walsh, N. C. et al. Humanized mouse models of clinical disease. *Annu. Rev. Pathol.* **12**, 187–215 (2017).
196. King, M., Pearson, T., Rossini, A. A., Shultz, L. D. & Greiner, D. L. Humanized mice for the study of type 1 diabetes and beta cell function. *Ann. N. Y. Acad. Sci.* **1150**, 46–53 (2008).
197. Shultz, L. D., Ishikawa, F. & Greiner, D. L. Humanized mice in translational biomedical research. *Nat. Rev. Immunol.* **7**, 118–130 (2007).

198. Gerace, D. et al. Engineering human stem cell-derived islets to evade immune rejection and promote localized immune tolerance. *Cell Rep. Med.* **4**, 100879 (2023).
199. Vegas, A. J. et al. Long-term glycemic control using polymer-encapsulated human stem cell-derived beta cells in immune-competent mice. *Nat. Med.* **22**, 306–311 (2016).
200. Chen, Y. G., Mathews, C. E. & Driver, J. P. The role of NOD mice in type 1 diabetes research: lessons from the past and recommendations for the future. *Front. Endocrinol.* **9**, 51 (2018).
201. Burrack, A. L., Martinov, T. & Fife, B. T. T cell-mediated beta cell destruction: autoimmunity and alloimmunity in the context of type 1 diabetes. *Front. Endocrinol.* **8**, 343 (2017).
202. Serreze, D. V., Niens, M., Kulik, J. & DiLorenzo, T. P. in *Mouse Models for Drug Discovery (Series Ed. Walker, J. M. Methods in Molecular Biology Vol. 1438)* (eds Proetzel, G. & Wiles, M. V.) 137–151 (Springer, 2016).
203. Takaki, T. et al. HLA-A*0201-restricted T cells from humanized NOD mice recognize autoantigens of potential clinical relevance to type 1 diabetes. *J. Immunol.* **176**, 3257–3265 (2006).
204. Radenković, M., Stojanović, M. & Prostran, M. Experimental diabetes induced by alloxan and streptozotocin: the current state of the art. *J. Pharmacol. Toxicol. Methods* **78**, 13–31 (2016).
205. Furman, B. L. Streptozotocin-induced diabetic models in mice and rats. *Curr. Protoc.* **1**, e78 (2021).
206. Gvazava, I. G., Rogovaya, O. S., Borisov, M. A., Vorotelyak, E. A. & Vasiliev, A. V. Pathogenesis of type 1 diabetes mellitus and rodent experimental models. *Acta Nat.* **10**, 24–33 (2018).
207. Bortel, R. et al. Levels of Art2$^+$ cells but not soluble Art2 protein correlate with expression of autoimmune diabetes in the BB rat. *Autoimmunity* **33**, 199–211 (2001).
208. Mordes, J. P., Bortell, R., Blankenhorn, E. P., Rossini, A. A. & Greiner, D. L. Rat models of type 1 diabetes: genetics, environment, and autoimmunity. *ILAR J.* **45**, 278–291 (2004).
209. Woda, B. A. & Padden, C. BioBreeding/Worcester (BB/Wor) rats are deficient in the generation of functional cytotoxic T cells. *J. Immunol.* **139**, 1514–1517 (1987).
210. Brehm, M. A., Powers, A. C., Shultz, L. D. & Greiner, D. L. Advancing animal models of human type 1 diabetes by engraftment of functional human tissues in immunodeficient mice. *Cold Spring Harb. Perspect. Med.* **2**, a007757 (2012).
211. Gonzalez, B. J., Creusot, R. J., Sykes, M. & Egli, D. How safe are universal pluripotent stem cells? *Cell Stem Cell* **26**, 307–308 (2020).
212. Rongvaux, A. et al. Human hemato-lymphoid system mice: current use and future potential for medicine. *Annu. Rev. Immunol.* **31**, 635–674 (2013).
213. Brehm, M. A. et al. Human immune system development and rejection of human islet allografts in spontaneously diabetic NOD-*Rag1*^{null} *IL2rγ*^{null} *Ins2*^{Akita} mice. *Diabetes* **59**, 2265–2270 (2010).
214. Tan, S. et al. Type 1 diabetes induction in humanized mice. *Proc. Natl Acad. Sci. USA* **114**, 10954–10959 (2017).
215. Ellis, C. E. et al. Human A2-CAR T cells reject HLA-A2$^+$ human islets transplanted into mice without inducing graft-versus-host disease. *Transplantation* **107**, e222–e233 (2023).
216. Balboa, D., Iworima, D. G. & Kieffer, T. J. Human pluripotent stem cells to model islet defects in diabetes. *Front. Endocrinol.* **12**, 642152 (2021).
217. Doloff, J. C. et al. Identification of a humanized mouse model for functional testing of immune-mediated biomaterial foreign body response. *Sci. Adv.* **9**, eade9488 (2023).
218. Li, Y. et al. Humanized mice reveal new insights into the thymic selection of human autoreactive CD8$^+$ T cells. *Front. Immunol.* **10**, 63 (2019).
219. Renner, S. et al. Permanent neonatal diabetes in *INS*^{C94Y} transgenic pigs. *Diabetes* **62**, 1505–1511 (2013).
220. Pabst, R. The pig as a model for immunology research. *Cell Tissue Res.* **380**, 287–304 (2020).
221. Casu, A. et al. Metabolic aspects of pig-to-monkey (*Macaca fascicularis*) islet transplantation: implications for translation into clinical practice. *Diabetologia* **51**, 120–129 (2008).
222. Pepper, A. R. et al. Establishment of a stringent large animal model of insulin-dependent diabetes for islet autotransplantation: combination of pancreatectomy and streptozotocin. *Pancreas* **42**, 329–338 (2013).
223. Kin, T., Korbutt, G. S., Kobayashi, T., Dufour, J. M. & Rajotte, R. V. Reversal of diabetes in pancreatectomized pigs after transplantation of neonatal porcine islets. *Diabetes* **54**, 1032–1039 (2005).
224. Smink, A. M. et al. Successful islet transplantation into a subcutaneous polycaprolactone scaffold in mice and pigs. *Transpl. Direct* **9**, e1417 (2023).
225. Gibly, R. F. et al. Extrahepatic islet transplantation with microporous polymer scaffolds in syngeneic mouse and allogeneic porcine models. *Biomaterials* **32**, 9677–9684 (2011).
226. Kim, S. et al. Molecular and genetic regulation of pig pancreatic islet cell development. *Development* **147**, dev186213 (2020).
227. Knechtle, S. J., Shaw, J. M., Hering, B. J., Kraemer, K. & Madsen, J. C. Translational impact of NIH-funded nonhuman primate research in transplantation. *Sci. Transl. Med.* **11**, eaau0143 (2019).
228. Dehoux, J. P. & Gianello, P. The importance of large animal models in transplantation. *Front. Biosci.* **12**, 4864–4880 (2007).
229. Graham, M. L. & Schuurman, H. J. Validity of animal models of type 1 diabetes, and strategies to enhance their utility in translational research. *Eur. J. Pharmacol.* **759**, 221–230 (2015).
230. Fitch, Z. et al. Transplant research in nonhuman primates to evaluate clinically relevant immune strategies in organ transplantation. *Transpl. Rev.* **33**, 115–129 (2019).
231. Brennan, F. R. et al. Safety testing of monoclonal antibodies in non-human primates: case studies highlighting their impact on human risk assessment. *MAbs* **10**, 1–17 (2018).
232. Coe, T. M., Markmann, J. F. & Rickert, C. G. Current status of porcine islet xenotransplantation. *Curr. Opin. Organ. Transpl.* **25**, 449–456 (2020).
233. Graham, M. L. et al. Clinically available immunosuppression averts rejection but not systemic inflammation after porcine islet xenotransplant in cynomolgus macaques. *Am. J. Transpl.* **22**, 745–760 (2022).
234. Sykes, M. & Sachs, D. H. Progress in xenotransplantation: overcoming immune barriers. *Nat. Rev. Nephrol.* **18**, 745–761 (2022).
235. Kirk, A. D. Crossing the bridge: large animal models in translational transplantation research. *Immunol. Rev.* **196**, 176–196 (2003).
236. D'Amour, K. A. et al. Efficient differentiation of human embryonic stem cells to definitive endoderm. *Nat. Biotechnol.* **23**, 1534–1541 (2005).
237. D'Amour, K. A. et al. Production of pancreatic hormone-expressing endocrine cells from human embryonic stem cells. *Nat. Biotechnol.* **24**, 1392–1401 (2006).
238. Nair, G. G. et al. Recapitulating endocrine cell clustering in culture promotes maturation of human stem-cell-derived β cells. *Nat. Cell Biol.* **21**, 263–274 (2019).
239. Breakthrough T1D. Cures program research strategy. *BreakthroughT1D.org* https://www.breakthrought1d.org/wp-content/uploads/2024/05/Breakthrough-T1D-Research-Strategy-Cures-1.pdf (2024).
240. Lu, K., Brauns, T., Sluder, A. E., Poznansky, M. C. & Dogan, F. Combinatorial islet protective therapeutic approaches in β-cell transplantation: rationally designed solutions using a target product profile. *FASEB Bioadv* **5**, 287–304 (2023).
241. European Commission. *Diabetes Reversing Implants with Enhanced Viability and Long-term Efficacy* (CORDIS, 2015).
242. European Commission. *Beta Cell Generation By Stem Cell-derived Implants In Diabetes* (CORDIS, 2015).
243. Breakthrough T1D. Breakthrough T1D Beta Cell Replacement Consortium: sharing data and resources, saving time and money. *BreakthroughT1D.org* https://www.breakthrought1d.org/news-and-updates/jdrf-beta-cell-replacement-consortium-sharing-data-resources-saving-time-money/ (2019).
244. US Food and Drug Administration. FDA approves first cellular therapy to treat patients with type 1 diabetes. *FDA* www.fda.gov/news-events/press-announcements/fda-approves-first-cellular-therapy-treat-patients-type-1-diabetes (2023).

Acknowledgements

A.G. is supported by NIH NIDDK R01DK132104, R01DK133610, JDRF 2-SRA-2022-1224-S-B, JDRF 2-SRA-2021-1078-S-B, Vivian Smith Foundation and Men of Distinction. A.R.P. is supported through a JDRF Career Development Award (5-CDA-2020-945-A-N) and is a Canada Research Chair in Cell Therapies for Diabetes thanks to funding from the Canada Research Chairs Program. A.C. is supported by a grant from JDRF (3-SRA-2022-1155-S-B) and the Italian Ministry of Health (GR-2018-12366399). M.B. is a consultant for The Jackson Laboratory. J.R.M. was supported by the NIH (R01DK114233), JDRF (3-SRA-2023-1295-S-B), and the Edward J Mallinckrodt Foundation. H.A.R. is or was supported by NIDDK R01DK12044, NIDDK R01DK132387, NINDS 1R01NS122911, NIDDK/HIRN RRID: SCR_014393; UC24 DK104162, JDRF SRA 2-SRA-2023-1313-S-B and 3-SRA-2023-1367-S-B, and the Diabetes Research Connection. M.C.P. is supported by JDRF grants 2-SRA 2021 1075-S-B and 3-SRA 2023 1365-S-B, the VIC Innovation Fund and The Hill Family Foundation. F. Dogan helped to create Fig. 2. The authors thank S. P. Rodgers and R. E. Whitehead for their support in finalizing the manuscript and figures.

Author contributions

A.G., N.E.M., J.G., M.C.P. and P.deV. researched data for the article, made a substantial contribution to discussion of content, wrote, and reviewed/edited the manuscript before submission. G.K., A.A.T., A.J.G., A.R.P., C.S., M.B., K.P., A.C., H.S., J.R.M., J.M.-M., M.G., M. Sefton, M. Ma, N.K., O.V., T.A.D., M.C.N., M. Marinac, M. Sykes, H.A.R., J.O. and Q.T. researched data for the article, made a substantial contribution to discussion of content and wrote the article. C.R. and E.L. wrote, and reviewed/edited the manuscript before submission.

Competing interests

A.G. is a co-founder of Continuity Biosciences LLC, and an inventor of intellectual property licensed by the same company. A.J.G. is an inventor of intellectual property related to technologies for cell therapy in T1DM owned in part by the Georgia Tech Research Corporation, is a co-founder, sits on the Board of Directors, and owns equity interest in iTolerance Inc. H.S. is an inventor on a patent licensed by iTolerance Inc, is a co-founder of the Company, and serves on the scientific advisory board of the Company. M. Ma is a co-founder and equity holder of AvantGuard and Persista Bio. J.O. is co-founder, owns stock equity, and serves on the scientific advisory board of Regenerative Medical Solutions Inc., is a clinical trial investigator for Vertex Pharmaceuticals Inc., and is a member of DSMB for Sernova Corp. J.R.M. is an inventor on related patents and patent applications, was employed at Sana Biotechnology, and has stocks and options in Sana Biotechnology. T.A.D. is a scientific founder of Encellin Inc., a cell therapy device company. K.P. discloses interest in Procyon Technologies LLC. M.C.N. has a sponsored research agreement with Universal Cells Inc., and a patent licensed to Sernova Corp. H.A.R. holds patents in the regenerative medicine space and served as SAB member of Sigilon Therapeutics, Prellis Biologics and consults or consulted for Sigilon Therapeutics, Eli Lilly, Minutia, Guidepoint Global, Axon Advisors and Tolerance Bio. C.R. is scientific adviser to Novo Nordisk, Vertex Pharma and iTolerance, and is a founding scientist of Lipogems International and AION Healthspan. M.C.P. is scientific founder of Vicapsys Life Sciences Inc. All other authors declare no competing interests.

Additional information
Peer review information *Nature Reviews Endocrinology* thanks Emmanuel Opara, Adrian Teo, Shareen Forbes for their contribution to the peer review of this work.

Alessandro Grattoni[1,2,3] ✉, Gregory Korbutt[4,5], Alice A. Tomei[6,7,8,9], Andrés J. García[10], Andrew R. Pepper[5], Cherie Stabler[11,12], Michael Brehm[13], Klearchos Papas[14], Antonio Citro[15], Haval Shirwan[16], Jeffrey R. Millman[17,18], Juan Melero-Martin[19,20,21], Melanie Graham[22,23], Michael Sefton[24,25], Minglin Ma[26], Norma Kenyon[6,8], Omid Veiseh[27], Tejal A. Desai[28,29], M. Cristina Nostro[30,31], Marjana Marinac[32], Megan Sykes[33,34,35], Holger A. Russ[12,36], Jon Odorico[37,38], Qizhi Tang[39,40,41], Camillo Ricordi[6,8], Esther Latres[42], Nicholas E. Mamrak[42,45], Jaime Giraldo[42,45] ✉, Mark C. Poznansky[43,45] ✉ & Paul de Vos[44,45] ✉

[1]Department of Nanomedicine, Houston Methodist Research Institute, Houston, TX, USA. [2]Department of Surgery, Houston Methodist Hospital, Houston, TX, USA. [3]Department of Radiation Oncology, Houston Methodist Hospital, Houston, TX, USA. [4]Alberta Diabetes Institute, University of Alberta, Edmonton, Alberta, Canada. [5]Department of Surgery, University of Alberta, Edmonton, Alberta, Canada. [6]Diabetes Research Institute, University of Miami Miller School of Medicine, Miami, FL, USA. [7]Department of Biomedical Engineering, University of Miami, Miami, FL, USA. [8]Department of Surgery, University of Miami Miller School of Medicine, Miami, FL, USA. [9]Department of Microbiology and Immunology, University of Miami Miller School of Medicine, Miami, FL, USA. [10]Woodruff School of Mechanical Engineering and Petit Institute for Bioengineering and Bioscience, Georgia Institute of Technology, Atlanta, GA, USA. [11]J. Crayton Pruitt Family Department of Biomedical Engineering, Herbert Wertheim College of Engineering, University of Florida, Gainesville, FL, USA. [12]Diabetes Institute, University of Florida, Gainesville, FL, USA. [13]Program in Molecular Medicine, Diabetes Center of Excellence, University of Massachusetts Chan Medical School, Worcester, MA, USA. [14]Department of Surgery, The University of Arizona, Tucson, AZ, USA. [15]Diabetes Research Institute, IRCCS Ospedale San Raffaele, Milan, Italy. [16]Department of Pediatrics, Ellis Fischel Cancer Center, School of Medicine, University of Missouri, Columbia, MO, USA. [17]Division of Endocrinology, Metabolism and Lipid Research, Washington University School of Medicine, St. Louis, MO, USA. [18]Department of Biomedical Engineering, Washington University in St. Louis, St. Louis, MO, USA. [19]Department of Cardiac Surgery, Boston Children's Hospital, Boston, MA, USA. [20]Department of Surgery, Harvard Medical School, Boston, MA, USA. [21]Harvard Stem Cell Institute, Cambridge, MA, USA. [22]Department of Surgery, University of Minnesota, Minneapolis, MN, USA. [23]Department of Veterinary Population Medicine, University of Minnesota, St. Paul, MN, USA. [24]Institute of Biomedical Engineering, University of Toronto, Toronto, Ontario, Canada. [25]Department of Chemical Engineering and Applied Chemistry, University of Toronto, Toronto, Ontario, Canada. [26]Department of Biological and Environmental Engineering, Cornell University, Ithaca, NY, USA. [27]Department of Bioengineering, Rice University, Houston, TX, USA. [28]University of California, San Francisco, Department of Bioengineering and Therapeutic Sciences, San Francisco, CA, USA. [29]Brown University, School of Engineering, Providence, RI, USA. [30]McEwen Stem Cell Institute, University Health Network, Toronto, ON, Canada. [31]Department of Physiology, University of Toronto, Toronto, ON, Canada. [32]Advocacy Department, Breakthrough T1D, Washington, DC, USA. [33]Department of Medicine, Columbia Center for Translational Immunology, Columbia University, New York, NY, USA. [34]Department of Microbiology and Immunology, Columbia University, New York, NY, USA. [35]Department of Surgery, Columbia University, New York, NY, USA. [36]Department of Pharmacology and Therapeutics, University of Florida, Gainesville, FL, USA. [37]UW Health Transplant Center, Madison, WI, USA. [38]Division of Transplantation, Department of Surgery, University of Wisconsin School of Medicine and Public Health, Madison, WI, USA. [39]Diabetes Center, University of California San Francisco, San Francisco, CA, USA. [40]Department of Surgery, University of California San Francisco, San Francisco, CA, US. [41]Gladstone Institute of Genomic Immunology, University of California San Francisco, San Francisco, CA, USA. [42]Research Department, Breakthrough T1D, New York, NY, USA. [43]Vaccine and Immunotherapy Center, Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA. [44]Immunoendocrinology, Division of Medical Biology, Department of Pathology and Medical Biology, University of Groningen and University Medical Center Groningen, Groningen, Netherlands. [45]These authors contributed equally: Nicholas E. Mamrak, Jaime Giraldo, Mark C. Poznansky, Paul de Vos.