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Engineering β Cell Replacement Therapies for Type 1 Diabetes: Biomaterial Advances and Considerations for Macroscale Constructs

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Abstract

While significant progress has been made in treatments for type 1 diabetes (T1D) based on exogenous insulin, transplantation of insulin-producing cells (islets or stem cell–derived β cells) remains a promising curative strategy. The current paradigm for T1D cell therapy is clinical islet transplantation (CIT)—the infusion of islets into the liver—although this therapeutic modality comes with its own limitations that deteriorate islet health. Biomaterials can be leveraged to actively address the limitations of CIT, including undesired host inflammatory and immune responses, lack of vascularization, hypoxia, and the absence of native islet extracellular matrix cues. Moreover, in efforts toward a clinically translatable T1D cell therapy, much research now focuses on developing biomaterial platforms at the macroscale, at which implanted platforms can be easily retrieved and monitored. In this review, we discuss how biomaterials have recently been harnessed for macroscale T1D β cell replacement therapies.

1. INTRODUCTION

Type 1 diabetes (T1D) is a chronic disease caused by the autoimmune destruction of insulin-producing β cells, located in clusters called islets of Langerhans in the pancreas. The irreversible destruction of functional β cell mass manifests as insufficient insulin levels and loss of glycemic control. When glycemic levels are left unmanaged, T1D patients face possible consequences of permanent organ damage (1) and secondary complications, such as neuropathy, retinopathy, nephropathy, and cardiovascular disease (2).

Current clinical treatment modalities are based on lifelong, daily exogenous insulin therapy and frequent blood glucose measurements. Since the discovery of insulin in the 1920s, significant technical advances have assisted T1D patients in maintaining tight glycemic control: Insulin analogs now vary in onset, peak time, and duration (3); routes of administration range from subcutaneous injections and pump infusions to nasal sprays (4); and closed-loop systems exist, wherein glucose monitoring technologies are combined with automated insulin delivery (5). Notwithstanding such progress, T1D patient outcomes remain unstandardized and poor (6). Exogenous insulin treatments are associated with delayed kinetics, failing to recapitulate physiological levels of glucose response and insulin secretion. Furthermore, exogenous insulin treatments require high patient compliance and, thus, are inconvenient (5). Hence, cell therapies are a promising alternative, for they harness a β cell's inherent capability to produce and secrete endogenous insulin on demand.

One form of β cell replacement therapy (BCRT), clinical islet transplantation (CIT), involves the infusion of human cadaveric donor islets through the hepatic portal vein. The Edmonton Protocol sparked enthusiasm for CIT in 2000, when seven out of seven patients, each receiving islets from an average of two human donor pancreata [~11,500 islet equivalents (IEQ)/kg of body weight], demonstrated independence from exogenous insulin for an average of 12 months (1, 7). IEQ is the standard measurement for counting islets; one IEQ equals one spherical islet of 150 μm in diameter. The Clinical Islet Transplantation Consortium, comprising 13 clinical centers worldwide, was established to enhance CIT's safety and long-term success. A multicenter, single-arm, phase 3 trial conducted by this consortium (NCT00434811) demonstrated restoration of hypoglycemia awareness and that 87.5% of the 48 adult diabetic subjects met the primary end point, HbA_{1c} (a measure of long-term glucose levels) <7.0%, after 1 year (8). As of 2017, approximately 1,500 patients have undergone islet transplantation, and 50–70% of patients have maintained insulin independence (i.e., independence from exogenous insulin) at 5 years (9).

Whereas substantial progress in islet isolation protocols, intraportal surgical procedures, and immunosuppressive drug regimens has positioned CIT as a promising therapeutic modality (9), several significant limitations restrict CIT to a marginal subset of T1D patients. First, the required systemic immunosuppression of CIT is associated with morbidities, which then limit CIT to the most critical cases of T1D—defined as frequent hypoglycemia and unstable metabolic control (10). Second, a scarcity of quality human donor pancreata exists, especially because multiple donors may be required for each recipient under current CIT protocols (9). Third, high early loss of islets (>60%) and long-term islet dysfunction are expected due to the suboptimal nature of the liver transplant site (11).

Biomaterials have the potential to revolutionize BCRTs by addressing current limitations. A subset of BCRT research focuses on nano- and microscale BCRT technologies, which, respectively, coat individual islets and encapsulate individual islets in microsized capsules (diameter <1 mm) (12, 13). Upon implantation, nano- and microscale BCRT technologies are virtually irretrievable, challenging to monitor, and likely to clump in body cavities (14). In contrast, macroscale BCRT technologies confer retrievability. All therapeutic cargo is confined and transplanted in one

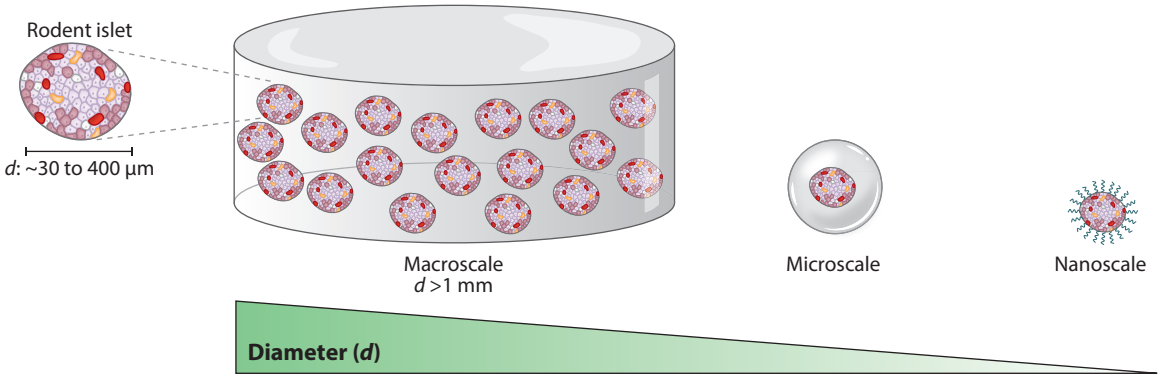

Figure 1

Size range of current β cell replacement therapies: macro-, micro-, and nanoscale technologies. Each rodent islet ranges from ~30 to 400 μm in diameter. Macroscale technologies confine many islets in a defined site, either in a hydrogel or scaffold (diameter >1 mm). Microscale technologies enclose each islet in a microsized capsule. Nanoscale technologies coat each islet.

or multiple defined site(s), such that facile implantation and retrieval procedures may be possible. **Figure 1** summarizes the size range of current BCRTs.

Retrievability has escalated as a critical clinical attribute with the advent of alternative cell sources such as xenogeneic porcine islets and human stem cell–derived β (SC-β) cells. Xenogeneic porcine islets are a promising alternative cell source, given that porcine insulin differs from human insulin by a single amino acid (15) and pigs are readily available with farming techniques. Indeed, Ludwig et al. (16) have demonstrated the practicality of using porcine islets in BCRTs. SC-β cells are a replenishable, renewable source for BCRTs (17). Two major companies have initiated clinical trials with SC-β cells; these trials will provide significant insight into the feasibility of using SC-β cells in humans. ViaCyte, the first company to introduce SC-β cells to treat T1D in clinical trials, has multiple ongoing clinical trials for its proprietary SC-β cells (NCT02239354, NCT02939118, NCT04678557, NCT03163511, and NCT03162926). In the ViaCyte clinical trials, the SC-β cells are allowed to differentiate and mature in vivo. In March 2021, Vertex announced its phase I/II clinical trial for VX-880, the first fully differentiated SC-β cells to treat T1D; VX-880 will be infused into the hepatic portal vein of patients under systemic immunosuppression (NCT04786262).

While xenogeneic porcine islets and SC-β cells may provide a virtually unlimited therapeutic cell supply, several safety concerns exist. A major concern with porcine islets is the possible infectious risk associated with the transmission of porcine endogenous viruses, though efforts in genome engineering and CRISPR-Cas9 aim to minimize this concern (18). Subpopulations of undifferentiated SC-β cells can form teratomas in vivo, and undesired polyhormonal, heterogeneous populations may result during differentiation (19). The concerns of using SC-β cells may be addressed by the outcome of Vertex's clinical trial (NCT04786262). In the meantime, until these safety concerns are addressed, macroscale BCRTs must prevent undifferentiated cell escape by incorporating safety precautions, such as complete retrieval capabilities.

Herein, we review current biomaterial technologies under development for macroscale T1D BCRTs. First, we address the fundamentals: We begin with native islet microarchitecture and CIT, specifically looking at what aspects of CIT present challenges to islet viability and function. Although the focus is on islet transplantation, the underlying issues are also relevant to human SC-β cells and xenogeneic insulin-producing cells. We then provide a rationale for, a definition of, and the types of macroscale BCRTs. Next, a detailed summary of pertinent biomaterial advances is presented, highlighting four areas: (*a*) mitigation of the host inflammatory and immune responses,

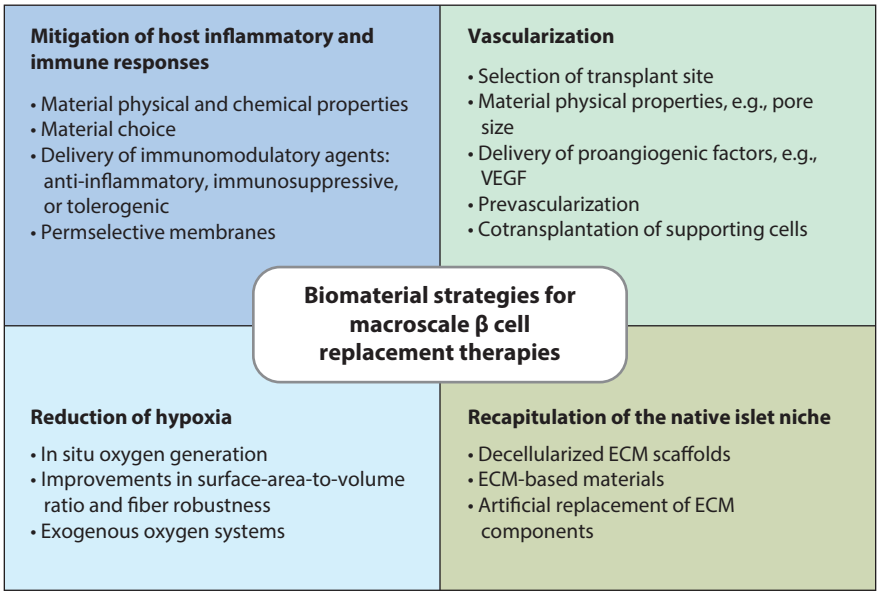

Figure 2

Overview of biomaterial strategies for macroscale β cell replacement therapies: mitigation of host inflammatory and immune responses, vascularization, reduction of hypoxia, and recapitulation of the native islet niche. Abbreviations: ECM, extracellular matrix; VEGF, vascular endothelial growth factor.

(*b*) vascularization of transplanted cells, (*c*) reduction of hypoxia, and (*d*) recapitulation of the native islet niche (**Figure 2**). Finally, we discuss current clinical trials and provide future considerations for this evolving field.

2. FUNDAMENTALS: NATIVE ISLET STRUCTURE AND CLINICAL ISLET TRANSPLANTATION

To justify the biomaterial-mediated strategies discussed in the following sections, we provide the context for how CIT builds an inhospitable milieu, unlike the native islet microenvironment. This section briefly reviews vital components of the native islet architecture, consequences of islet isolation, and undesired host responses in the liver.

2.1. Native Islet Microarchitecture

The islet of Langerhans is a micro-organ that comprises various cell types: glucagon-producing α cells, insulin-producing β cells, somatostatin-producing δ cells, ghrelin-producing ε cells, and pancreatic polypeptide-producing cells. A rich microvasculature runs within and around each micro-organ. Islets receive approximately 15% of pancreatic blood flow while composing only 1–2% of total pancreatic mass (20). This microvasculature, which includes dense and fenestrated capillaries, delivers necessary signaling hormones, metabolites, and nutrients (such as glucose) to endocrine cells. It also initiates the systemic distribution of secreted hormones from endocrine cells. The result is glucose homeostasis: Islets can sensitively detect glucose and quickly secrete insulin on demand.

The rich intraislet blood flow also supplies a continuous oxygen supply, with a mean partial pressure of oxygen of 40 mm Hg (21). Moreover, islets have elevated oxygen consumption rates

compared with many other cells (22), and β cells have been shown to metabolize glucose nearly exclusively by aerobic glycolysis (i.e., oxidative metabolism) (23). Evidence suggests that islets exhibit up to a 50% loss in insulin secretory function under even minor oxygen tension variations and acute exposure to hypoxia (24).

The extracellular matrix (ECM) imparts critical biophysical and biochemical cues to islets. As an acellular component of the tissue microenvironment, the ECM comprises polysaccharides (glycosaminoglycans) and proteins (fibronectin, collagens, and laminins) (25). For islets, the ECM is found in the basement membrane that surrounds capillaries and in the interstitial ECM between islet cells. Integrin transmembrane receptors bind to ECM molecules, thereby activating intracellular pathways that ultimately regulate cell activities. For instance, the $\alpha_1\beta_1$-collagen IV interaction upregulates metabolic function and inhibits apoptosis; also inhibiting apoptosis are the $\alpha_v\beta_3$-laminin and $\alpha_5\beta_1$-fibronectin interactions, via Bcl-2 activation (25). The $\alpha_6\beta_1$-laminin interaction regulates insulin release (26).

2.2. Islet Isolation Procedures Inevitably Destroy Native Islet Microarchitecture

Although necessary for BCRT, isolation procedures destroy native islet microarchitecture. Islet isolation techniques include mechanical disruption and enzymatic digestion. The result is the separation of endocrine tissue from exocrine tissue. Inevitably, islets are left avascular, ischemic, denervated, and detached from cellular contacts and the endogenous ECM (27). Isolated islets can then undergo anoikis, cell death induced by detachment from the ECM (28). A recent report suggests that focal adhesion activation in β cells exclusively occurs where β cells contact the capillary ECM (29), underscoring the need for rapid revascularization.

2.3. The Liver Is an Inhospitable Transplant Site for Islets

Although clinically accessible, the liver transplant site further contributes to islet dysfunction and death. Complete revascularization of islets may take upward of 14 days (30). Thus, islets must rely on passive diffusion of nutrients, thereby undergoing a transient hypoxia period and delayed kinetics of nutrients. Exposure to hypoxia causes islets to produce danger-associated molecular patterns (31); generate nitric oxide (32); upregulate proinflammatory cytokines such as monocyte chemoattractant protein (33); and activate hypoxia-inducible factor-1α (HIF-1α)-related pathways that stimulate inflammatory pathways, impair glucose responsiveness, and terminate in apoptosis (34). Additionally, direct contact with blood within the hepatic vasculature triggers an instant blood-mediated inflammatory reaction (IBMIR), leading to complement activation and leukocyte infiltration (35). Due to IBMIR, 60% of transplanted islet mass is lost within the first few days of transplantation (36). Alloreactivity and autoimmunity further exacerbate the host immune response. Moreover, studies indicate that the systemic immunosuppressive drugs used in CIT—aimed to lessen the impacts of IBMIR, alloreactivity, and autoimmunity—also lead to islet dysfunction (37). In summary, in CIT, a high loss of functional islets is expected due to the immunologic, anatomic, and physiologic factors of CIT and the liver transplant site.

3. RATIONALE AND DEFINITION OF RETRIEVABLE, MACROSCALE β CELL REPLACEMENT THERAPIES

Macroscale BCRTs can be categorized as either closed, cell-restrictive systems or open, cell-permissive systems, depending on if the platform encourages host cell and vessel infiltration (**Figure 3**). Both approaches have advantages and disadvantages.

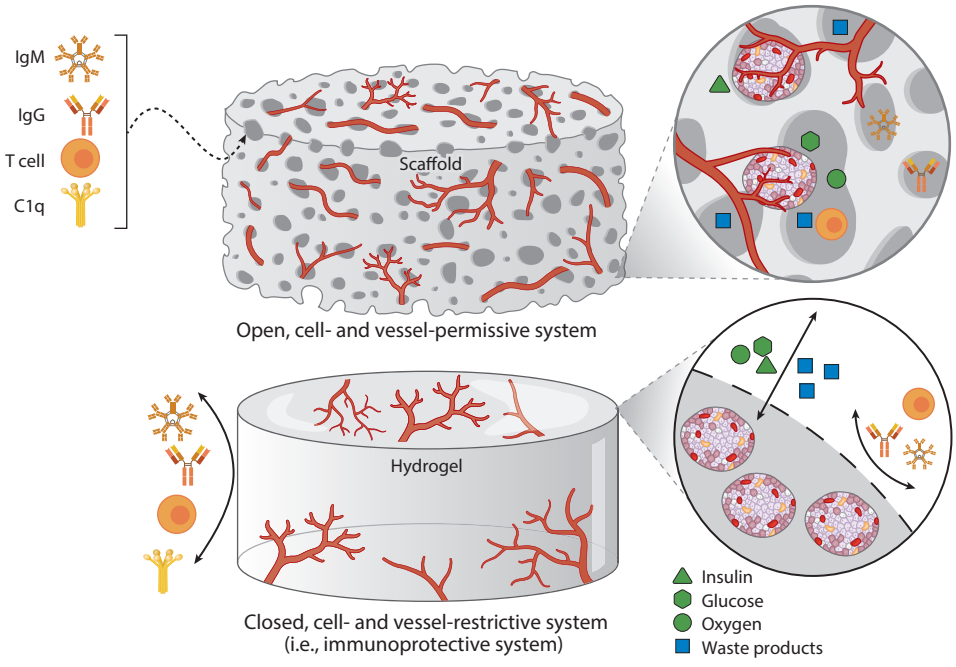

Figure 3

Types of macroscale β cell replacement therapies: open (cell- and vessel-permissive) and closed (cell- and vessel-restrictive) systems. Open systems have pores or channel diameters large enough to allow vessels and host cells—including host immune mediators such as antibodies, T cells, and C1q—to infiltrate the biomaterial platform and, thus, allow direct integration of grafted cells with the recipient. Closed systems include permselective membranes that physically prevent the entry of large host immune mediators and allow the bidirectional transport of necessary small molecules such as insulin, glucose, and oxygen. Abbreviations: C1q, complement component 1q; IgG, immunoglobulin G; IgM, immunoglobulin M.

Closed systems are designed on the principle of immunoprotection, the physical sequestration of donor cells from host cells by permselective membranes. Primarily on the basis of size exclusion, permselective membranes prevent the entry of large host immune mediators such as T cells and antibodies yet allow bidirectional transport of necessary small molecules such as insulin and oxygen (10). Closed, immunoprotective systems are further categorized as intravascular or extravascular. The former involve direct anastomosis of therapeutic platforms to host vasculature and are historically associated with high bleeding and infection rates (38, 39). On the other hand, extravascular devices have shown high promise and have even progressed to clinical trials. While we recognize that some intravascular macroencapsulation devices have been developed (40), we focus our review on extravascular macroencapsulation devices. Although closed systems inevitably cause mass transfer limitations of necessary nutrients to islets, closed systems reduce and may eliminate the need for immunosuppression.

Conversely, open systems allow direct integration of grafted cells with the recipient but require, at a minimum, some immunomodulation or immunosuppression. Traditional open systems are 3D biomaterial constructs with pores or channel diameters large enough for cell and vessel infiltration (41). Macroporous scaffolds are characterized by pores larger than 50 μm (42). Recently, deviceless (DL) platforms have emerged as open technologies. In a DL platform, a biomaterial construct loaded with no therapeutic cargo is first implanted at the transplant site. After prevascularization is achieved, the construct is removed and replaced with a cell-only graft (27).

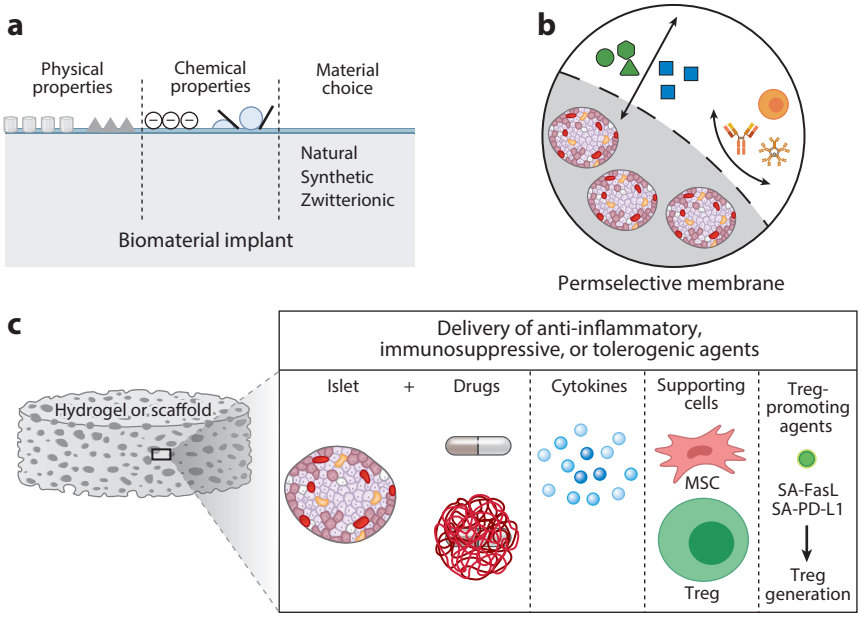

Figure 4

Approaches for the mitigation of host inflammatory and immune responses. (*a*) Material physical and chemical properties (e.g., surface topography, surface charge, and degree of hydrophilicity) and material choice. (*b*) Permselective membranes, through which insulin, glucose, oxygen, and waste products (shown in *green* and *blue*) may traverse freely, but host immune cells such as T cells and antibodies (shown in *orange*) cannot. (*c*) Delivery of anti-inflammatory, immunosuppressive, or tolerogenic agents from biomaterials. Abbreviations: MSC, mesenchymal stem cell; SA-FasL, streptavidin-modified Fas ligand; SA-PD-L1, streptavidin/programmed cell death-ligand 1; Treg, T regulatory cell.

4. BIOMATERIAL STRATEGIES FOR MACROSCALE β CELL REPLACEMENT THERAPIES

4.1. Mitigation of Host Inflammatory and Immune Responses

This section discusses strategies to mitigate the host inflammatory and immune responses to BCRTs (**Figure 4**).

4.1.1. Mitigation of fibrosis. Virtually all implants instigate the foreign body response (FBR) (see 43 for a comprehensive review on FBR). The development of fibrotic capsules surrounding implants over time can negatively impact in vivo therapeutic platforms, especially β cell–delivering macroencapsulation devices that rely on the diffusive transfer of crucial nutrients. Indeed, the clinically tested macroencapsulation devices PEC-Encap and βAir demonstrated acceptable safety profiles, preserved islet viability, and mechanical robustness (NCT02239354, NCT02939118, and NCT02064309); however, unfortunately, they also showed reduced overall graft efficacy due to strong FBR between host tissue and the synthetic membranes (44).

Promisingly, the degree of FBR may be regulated by the mechanical and chemical properties of biomaterials. To reduce fibrosis via biomaterial microarchitecture, researchers may consider smooth, well-contoured edges over sharp edges due to less shear stress between tissues and biomaterials (44). Also, soft biomaterial surfaces may be used over rigid surfaces due to less difference in stiffness between soft tissue and biomaterials (44). In terms of chemical properties, it is generally

accepted that protein adsorption at the host tissue-material interface and the subsequent cellular response are significant drivers of FBR. Hydrogels, which are highly hydrophilic materials, are commonly used for BCRTs given their high water content, mechanical properties similar to soft tissue, ease of fabrication, and biofunctionalization (44). Markedly, some researchers have harnessed, rather than mitigated, the early fibrotic response to promote islet graft efficacy (discussed in the section titled Prevascularization).

The choice of material also influences FBR. Types of biomaterials used in BCRTs are primarily categorized as natural or synthetic. Recently, zwitterionic materials, which have separate negatively and positively charged groups, have shown promise in mitigating FBR (45).

Natural materials are extracted from biological sources via solvents and/or enzymes. Natural biomaterials are further categorized as polysaccharides (e.g., alginate, agarose), proteins (e.g., collagen, fibrin), or decellularized ECM scaffolds (e.g., decellularized pancreas). The most investigated natural material for BCRTs is alginate for its cytocompatibility, mild cross-linking conditions, availability, and low production cost (1, 44). Natural materials based on proteins or ECM scaffolds have inherent cell-binding sites that support cell attachment, migration, proliferation, and differentiation (13). Nevertheless, despite their resemblance to native tissue, natural biomaterials typically have low mechanical stability, manufacturing issues, and limited capacity for precise tuning of mechanical properties and degradation rates (13).

Standardization of purification practices is a top priority for users of natural materials, especially for researchers using alginate. Due to unstandardized purification protocols and batch-to-batch variability (e.g., varying mannuronic/guluronic ratios), alginate-based findings are difficult to compare and reproduce among groups working with different alginate preparations. Commercially available ultrapure-labeled alginate may still contain detectable levels of contaminants such as endotoxins, which can lead to fibrosis via fibroblast recruitment (46). Furthermore, batch characteristics influence gelation, stability, and stiffness of the material (47). Ongoing controversy surrounds a pivotal study that demonstrated that 500-μm-diameter alginate microcapsules exhibited more macrophage and fibroblast adhesion than 1,800-μm-diameter counterparts (48). Although this size-dependence mechanism was observed across a wide span of materials (alginate, glass, and ceramic, among others), other groups report no such fibrosis with 500-μm-diameter alginate microcapsules; Hu & de Vos (49) postulate that this discrepancy is due to differences in alginate purification.

Synthetic materials confer the advantages of high durability, stability, reproducibility, and tunability of mechanical and chemical properties (e.g., pore size and composition). Simple, facile biofunctionalization of synthetic materials is also possible through processes such as tethering of adhesive peptides (50). Synthetic biomaterials used in BCRTs are typically organic polymers, including polyethylene glycol (PEG), polydimethylsiloxane (PDMS), polylactic-coglycolic acid (PLGA), polycaprolactone (PCL), and polytetrafluoroethylene (PTFE). Some are typically employed as soft hydrogels; others are frequently used as rigid scaffolds (PDMS, PTFE, PCL, and PLGA). Some synthetic polymers, especially aliphatic polyesters, are known to gradually degrade via hydrolysis (51), while other synthetic materials require manufacturing processes or harsh solvents that are potentially harmful to cells.

In some cases, researchers have combined natural and synthetic biomaterials in a single platform. Beta-O_2 Technologies' βAir macroencapsulation device combines an alginate hydrogel and a gas-permeable PTFE membrane (52). Likewise, through 3D fiber deposition, Marchioli et al. (53) combined a ring-shaped PCL scaffold with an alginate hydrogel core. In the former example, the PTFE membrane provided enhanced mechanical stability along the host-material interface. In the latter, Marchioli et al. manufactured the PCL scaffold with highly precise pore

sizes to facilitate vascularization. In both cases, alginate hydrogels encapsulated islets to preserve islet viability and function.

Zwitterionic polymers have emerged as novel materials to mitigate fibrosis. These polymers incorporate zwitterions, molecules with equal numbers of cationic and anionic moieties, thereby presenting an overall neutral charge. Zwitterions drive the creation of hydration shells upon the material surface—achieving superhydrophilicity and strong electrostatically induced hydration. The result is a significant reduction of nonspecific protein adsorption and cell adhesion. Indeed, the Jiang group (45) reported that zwitterionic hydrogels prevented FBR for at least 3 months in mice. Zwitterionic polymers have recently been used in T1D applications. The use of a zwitterionic polymer to coat T1D continuous glucose monitors (CGMs) augmented the reliability of CGMs and significantly reduced noise (54). Incorporation of specific zwitterions onto alginate mitigated cellular overgrowth on microcapsules in a diverse array of animals (mice, dogs, and pigs). Importantly, delivery of xenogeneic islets in modified microcapsules achieved and maintained normoglycemia in four out of six diabetic mice for 200 days until retrieval (55). Zwitterionic polymers present a promising outlook for macroscale BCRTs, as ongoing work aims to enhance their stability and functionality (56).

4.1.2. Delivery of immunomodulatory agents: anti-inflammatory, immunosuppressive, or tolerogenic. In addition to selecting biomaterials that mitigate fibrosis, researchers have leveraged anti-inflammatory, immunosuppressive, or tolerogenic agents to support islet survival and function. Drugs and cytokines may be loaded onto BCRTs for soluble release or chemically conjugated to biomaterials with prescribed release kinetics. With biomaterials as delivery vehicles, immunomodulatory cells such as mesenchymal stem cells (MSCs) and T regulatory cells (Tregs) may be codelivered with islets. Compared with systemic infusion of immunosuppressive drugs for CIT, localized and controlled delivery from biomaterials decreases systemic burden and reduces off-target effects (57).

As previously mentioned, one of the major drawbacks of CIT is early posttransplant inflammation. Accordingly, anti-inflammatory drugs have been incorporated into BCRTs. Several anti-inflammatory drugs target macrophages, crucial players in modulating early inflammation posttransplant. Macrophages release inflammatory cytokines and reactive oxygen species that later activate cells of the adaptive immune system, such as antigen-presenting cells, helper T cells, and cytotoxic T cells (58).

Two types of anti-inflammatory, macrophage-targeting drugs are the glucocorticoid dexamethasone (Dex) and Clodrosome. Dex polarizes monocytes toward the anti-inflammatory macrophage phenotype (M2) while preserving migratory function for wound healing and angiogenesis (57). Clodrosome, based on liposomes and clodronate, depletes macrophages. Local delivery of Dex from macroporous PDMS scaffolds demonstrated dose-dependent effects in murine diabetes treatment (59). Low dosages of Dex (0.25% and 0.1%) promoted M2 macrophage polarization and suppressed inflammatory pathways within the first week of implantation, whereas high loadings (0.5% and 1%) precluded desired host cell and vessel infiltration, thereby negatively impacting islet engraftment. Normoglycemia (defined in this study as two consecutive readings of <200 mg/dL) was observed within 10 days of implantation in $>90\%$ of mice receiving low Dex. Clodrosome, upon subcutaneous codelivery with rat islets in Matrigel, depleted macrophages and assisted islet survival in 83% of recipient mice for >60 days (60).

Immunosuppressive drugs have likewise been incorporated into biomaterials for T1D treatment. FK506, an immunosuppressant routinely used for CIT, impairs helper T cell–mediated macrophage activation and cytotoxic T cell–mediated graft rejection by obstructing gene transcription of interleukin-2 (IL-2) (61). Subcutaneous injection of FK506-loaded PLGA

microparticles and xenogeneic islets in Matrigel elicited minimal immune cell infiltration and prompted glucose clearance rates comparable with those of nondiabetic mice at 2 and 4 weeks (62).

A significant drawback of biomaterial-mediated drug monotherapy is drug depletion over time. In the previous examples, Dex (at 18 and 45 μg per PDMS scaffold implant), Clodrosome (at 6.25 mg/kg), and FK506 (at 10 mg/kg) were depleted after 10, 14, and ∼25 days, respectively. While major strides have prolonged the duration of drug release—such as Farah et al.'s (63) compact, solvent-free crystal platform—it is unlikely that biomaterial-mediated drug monotherapy alone can adequately protect allo- and xenografts long term. Conversely, antigen-specific immune tolerance induction strategies have the potential to achieve indefinite graft acceptance (64, 65). Tregs may be the key to permanent graft acceptance. They sustain self-tolerance by suppressing effective immune cell types such as helper T cells or dendritic cells, either by direct cell contact or through secretion of immunosuppressive cytokines [e.g., IL-10 or transforming growth factor beta 1 (TGF-β1)]. In an autoimmune diabetes murine model, Graham et al. (65) found that localization of Tregs with islets in PLG scaffolds prevented autoimmune destruction of islets and prolonged islet graft survival. Intriguingly, in a subset of recipients that had their first grafts removed and then replaced with a second, Treg-free graft, normoglycemia was achieved for the remaining 43 days of the study, 140 days from the initial transplantation. Immunostaining revealed $Foxp3^+$ cells neighboring the first grafts in the abdominal fat and the second grafts in the kidney capsule, suggesting systemic tolerance.

As the isolation of Tregs could produce undesired heterogeneous T cell populations, strategies focused on the promotion of Treg generation have been explored. Our group demonstrated that cotransplantation of microgels (150 μm in diameter) presenting a chimeric form of FasL with allogeneic islets induced long-term immune acceptance of the graft and restored normoglycemia in a diabetic mouse model (64). The FasL-presenting biomaterial generated Tregs, which were required for allograft acceptance and function. Also, Coronel et al. (66) developed a biomaterial platform that presented chimeric streptavidin/programmed cell death-ligand 1 (SA-PD-L1) on the surface of biotin-PEG microgels and found augmented populations of Tregs and $CD4^+$T anergic cells in the transplantation microenvironment. Future work may focus on scaling this application to grafts delivered in macrosized constructs.

4.1.3. Permselective membranes. Immunoprotection via permselective membranes is a simple, effective way to overcome the CIT requirement of chronic immunosuppression. Ideally, desired elements (nutrients, glucose, insulin, oxygen, and waste products) must be able to traverse permselective membranes, while host immune mediators (immune cells, antibodies, and complement) should not. A rich body of literature suggests that elements of the former category typically have lower molecular weights than those of the latter. **Table 1** summarizes relevant molecule sizes (67, 68). Of note, insulin is a significantly larger molecule than glucose and, thus, undergoes higher

Table 1 Summary of molecule sizes relevant to type 1 diabetes β cell replacement therapies

Category	Molecule	Properties
Desired molecules	Glucose	180 Da; Stokes radius 0.4 nm
	Insulin	Monomer/hexamer: 5.8–34.2 kDa; 1.35–2.75 nm
Host immune mediators	Inflammatory cells	Approximately 10 μm
	IgG	150 kDa; Stokes radius 5.9 nm
	C1q	410 kDa
	IgM	910 kDa

Abbreviations: C1q, complement component 1q; IgG, immunoglobulin G; IgM, immunoglobulin M.

diffusional limits that influence its kinetics. Additionally, it is generally agreed that increased immunogenicity correlates to lower permeability (i.e., xenogeneic sources require lower effective exclusion sizes than allogeneic sources) (13, 57). While molecular weight cutoff (MWCO) has been used as a design parameter for permselective membranes, we recognize that diffusive molecules exhibit a range of molecular weights and that molecular weight does not always correlate to size, shape, and relative charge. Permeability and diffusion coefficients, dependent on material chemistry, may provide better insight into the design of permselective membranes (13, 69).

Importantly, host immune mediators can be similar in size to desired molecules and can traverse through immunoprotective membranes. Indeed, free radicals have molecular weights between oxygen and glucose, and cytokines are of comparable molecular weight to insulin, growth factors (GFs), and albumin (68). In encapsulation systems, which may have membranes that have MWCOs as low as 70 kDa, donor cells can shed antigens that can later traverse membranes and subsequently prime host antigen-presenting cells (70). In summary, on the basis of pore size exclusively, permselective membranes are effective against cell contact-mediated and complement-mediated cytotoxicity, yet ineffective against proinflammatory cytokines (71) and indirect T cell activation (70). Other approaches to prevent undesired host immune reactions must be considered, such as the aforementioned immunomodulatory approaches and modifications in biomaterial properties. Also, as discussed in future sections, developing a viable 3D microenvironment for transplanted cells can mitigate inflammatory signals elicited from transplanted cells.

4.2. Vascularization

This section examines approaches aimed to induce vascularization within and/or around BCRTs (**Figure 5**).

4.2.1. Selection of transplant site. Rapid reconstruction of robust, mature vasculature begins with the selection of an appropriate transplant site. Open and closed macroscale systems benefit from revascularization within and around the chosen platform, respectively. In either case, several factors are desired: First, as evidenced by the native pancreas, dense vasculature and portal drainage are important for nutrient and oxygen delivery, as well as insulin secretion responses to blood glucose levels (42). Second, the site should be clinically relevant; it should permit minimally invasive methods for graft implantation, monitoring, biopsy, replenishment, and retrieval (27).

Several sites are dismissed, as follows. The kidney capsule, which has provided encouraging results in rodents, remains undesirable as an islet transplant site for larger animals due to invasive surgery, risk of diabetic nephropathy, and limited space for transplant volumes (72). Similarly, some immune-privileged sites such as the thymus and anterior eye chamber lack the volume to support the sizeable loading requirements of macroscale BCRTs (73). Other extrahepatic sites have been investigated for islet transplantation, all with varying degrees of neovascularization and inflammatory responses (as investigated in 11): striated muscle (NCT02872571), bone marrow (NCT01722682), venous sac (74), small bowel mesentery (11), gastric submucosa (75), omentum (76) (NCT02213003), and the subcutaneous space (77–79).

In particular, the omentum/peritoneal cavity and subcutaneous space are employed in most of the studies reviewed herein. The omentum is an attractive transplant site for BCRT, given its arterial supply, accessibility, and, distinctly, venous drainage that facilitates physiological insulin secretion through the portal system (42). The murine surrogate to the clinically relevant omentum is the gonadal fat pad (FP). The subcutaneous space is an appealing transplant site due to its large surface area for nonmarginal transplant volumes and accessibility for implantation, monitoring, biopsy, and retrieval. While the subcutaneous space is not inherently well vascularized, major efforts currently aim to induce vascularization at the site (27, 80–82).

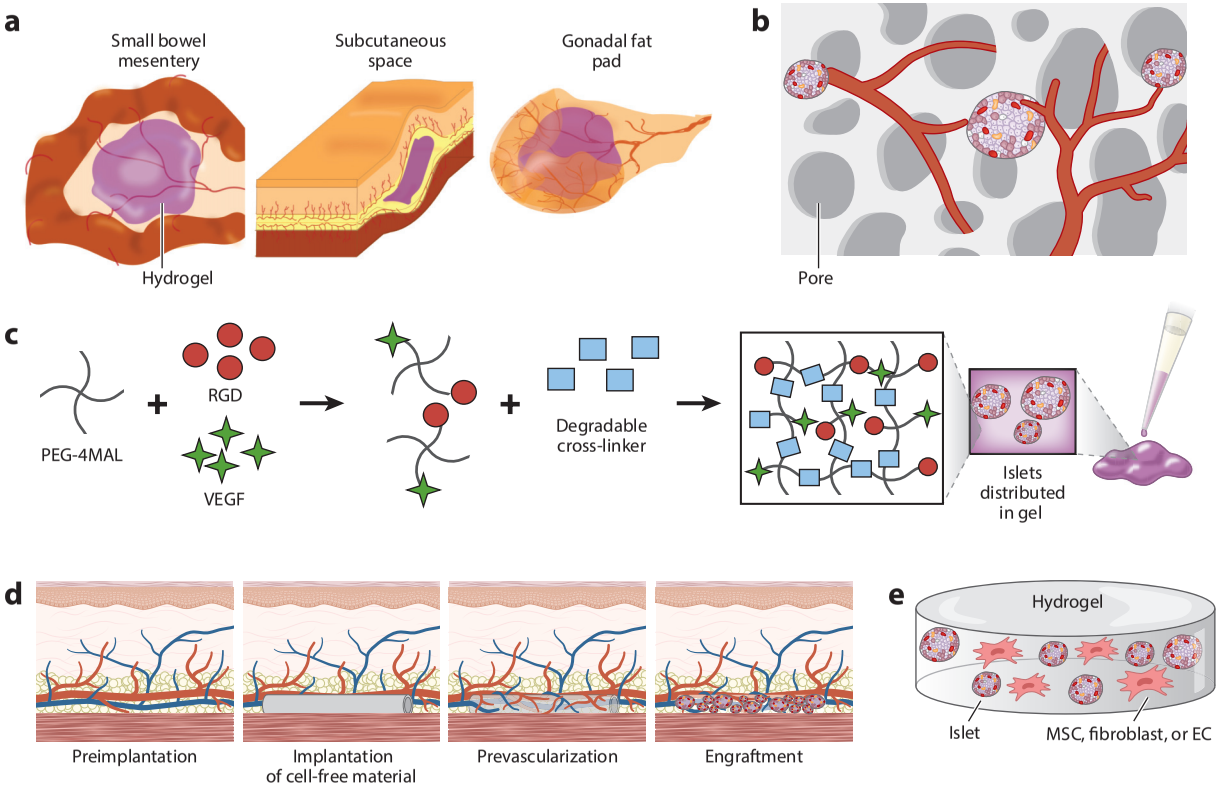

Figure 5

Approaches for vascularization. (*a*) Selection of an appropriate transplant site. (*b*) Material properties such as pore size. (*c*) Delivery of growth factors from biomaterials through mechanisms such as chemical conjugation. (*d*) Prevascularization, wherein a cell-free material is implanted for at least a week to achieve neovascularization before engraftment. (*e*) Codelivery of proangiogenic supporting cells. Panels *a* and *c* adapted with permission from Reference 11. Abbreviations: EC, endothelial cell; MSC, mesenchymal stem cell; PEG-4MAL, maleimide-terminated four-arm polyethylene glycol; RGD, arginine-glycine-aspartate; VEGF, vascular endothelial growth factor.

4.2.2. Physical properties of biomaterials. Physical properties of biomaterials, especially pore size, can prompt vasculogenic activity (28). In a seminal study, Brauker et al. (83) found that while pore diameters of 0.8–8 μm permitted host immune cell infiltration, this diameter range also was associated with increased numbers of vascular structures directly at the material-tissue interface. The study was conducted in the subcutaneous space of rats. Significantly, across four different synthetic commercial membranes, no intervening inflammatory cells populated the newly formed vasculature, demonstrating a reduction in fibrotic capsule thickness and indicating an altered foreign body reaction. A different conclusion was reached in a more recent study investigating cardiac implantation of acellular scaffolds; angiogenesis and reduced fibrotic response (as specified by a macrophage shift to the *M2* phenotype) were only associated with pore diameters of 30–40 μm (84). For porous PEG hydrogels, mature vascular ingrowth was marked for pore diameters of >100 μm following subfascial implantation in rats (85). For PLGA scaffolds transplanted in the subcutaneous space of mice, large blood vessels at low densities were observed only for pore diameters of >200 μm (86). Together, these studies suggest that the ideal pore size depends on the scaffold material and inherent vascular properties of the transplant site. However, specifically for BCRTs, studies support the use of platforms that limit the escape of islets approximately 30 to 400 μm in diameter (87). Regardless, investigation of pore-mediated vascularization has directly informed the designs of cell-permissive constructs and the designs of closed systems that incorporate cell-permissive outer layers adjacent to immunoprotective cores (53). While not discussed here, other parameter considerations besides pore size include porosity, surface roughness, window size, pore continuity, pore interconnectivity, and surface pore size (28, 86).

4.2.3. Delivery of proangiogenic factors. An elegant, facile strategy to promote neovascularization is the biomaterial-mediated delivery of proangiogenic factors. Such factors are conjugated to biomaterials or loaded into biomaterials for soluble release. Vascular endothelial growth factor (VEGF) has been commonly explored for BCRTs, as it promotes the growth of endothelial cells (ECs) and is a major regulator of native islet vascularization and development (88). In an alginate application, Gebe et al. (89) explored the synergistic potential of a macroporous polyvinyl alcohol scaffold (500-μm average pore size) and VEGF-releasing alginate core to prompt angiogenesis. Modest additions of VEGF (20 ng) released from the alginate core facilitated host microvessel infiltration into the surrounding scaffold (500-μm average pore size). Compared with VEGF-free constructs, VEGF-releasing constructs demonstrated a twofold increase in vascularity within the subcutaneous space and achieved a 30% time reduction to normoglycemia. Our group has employed VEGF in a two-part PEG-maleimide macroencapsulation platform, wherein the inner, nondegradable hydrogel (500 μL) encapsulated islets while the outer, degradable hydrogel released VEGF upon proteolytic degradation (90). Whole-mount imaging and lectin perfusion conducted 4 weeks posttransplant confirmed enhanced vascular ingrowth and density at the encapsulation material interface, which correlated with improved islet viability.

Other GFs, GF-recruitment strategies, and non-GF agents have been employed for graft revascularization. Fibrin hydrogels presenting platelet-derived growth factor (PDGF) functioned synergistically with macroporous PDMS scaffolds to increase vessel branching and mature intraislet vessels for a syngeneic diabetic mouse model (91). Likewise, heparin-releasing silk fibroin scaffolds improved syngeneic islet transplantation outcomes in diabetic mice, owing to heparin-mediated activation of endogenous VEGF/VEGFR2 pathways that promoted revascularization and proliferation (92). A minority of studies have employed non-GF angiogenic molecules, such as NECA (5′-(*N*-ethylcarboxamido)adenosine) released from PLGA sheets (93) and FTY720 released from PHBV-PCL membranes (94).

Collectively, these studies indicate that the reconstruction of robust vasculature is a complex process that many factors can influence. Interventions that focus on the delivery of proangiogenic cues must address issues associated with pharmacokinetics and must prevent the formation of leaky, dysfunctional vessels. Future macroscale BCRT studies should thoroughly investigate the synergistic effects of multiple GF codelivery or the sequential codelivery of GFs (95, 96). It has been suggested that some GFs, such as VEGF, may be more beneficial in the nascent stages of vascularization, while others, such as acidic fibroblast growth factor (FGF), are more suited to assist later stages of vascularization, which includes tube formation and vessel maturation (97).

4.2.4. Prevascularization. Prevascularization can reduce the time of revascularization at a selected transplant site. In this two-step strategy, a vascularization-inducing material is first implanted for a predetermined time, after which it is loaded with insulin-producing cells or completely removed to create a DL site. The FBR is used to generate a vascularized collagen network that is formed before mature fibrotic scars characteristic of a chronic inflammatory response (27).

Some prevascularization efforts employ biological agents. For example, after 1 week of prevascularization by agarose rods functionalized with basic FGF and heparin, the transplantation of 3,000 allogeneic rat islets in the dorsal subcutaneous space led to normoglycemia within 3 days and was sustained for >100 days (98). Therapeutic efficacy was accomplished with no immunosuppression and observed across three different allogeneic pairs of rat strains.

Other prevascularization strategies employ no biological factors. In one prevascularization method, scaffolds based on poly(D,L-lactide-coε-caprolactone) were each loaded with ∼3,500 islets from Albino Oxford rats and implanted in the dorsal subcutaneous space of Rowett nude rats for 28 days (99). Recipients of islet-loaded scaffolds in the prevascularized, subcutaneous site behaved comparably to positive controls (recipients of islets transplanted under the kidney capsule). Both groups achieved normoglycemia within 3 days and maintained such for the entire 16-week duration of the study.

In another approach, no materials are left behind following prevascularization. To create a prevascularized DL subcutaneous space in mice, Pepper et al. (27) implanted a nylon angiocatheter for 28 days. After catheter removal, 500 syngeneic islets were loaded into the resultant lumen. In 91% of DL recipients, normoglycemia was maintained for >100 days, and glucose clearance rates were comparable to those of kidney capsule recipients. This DL prevascularization method has since been used for human embryonic stem cell–derived pancreatic endoderm cells (PECs), eventually leading to diabetes correction in immunodeficient B6/Rag$^{-/-}$ mice (100). Unlike recipients of PECs in the unmodified subcutaneous space and recipients of PECs in the FP, recipients of PECs in the DL subcutaneous space demonstrated insulin independence at ∼100 days posttransplant and higher levels of human C-peptide at 8 and 12 weeks following transplantation. A biomarker for insulin production, C-peptide is a measure for graft efficacy and function.

Remarkably, prevascularization can reduce the curative dosage of islets required for diabetes reversal. Building upon previous work that demonstrated methacrylic acid (MAA)-based biomaterials could generate vessels in rodent models by insulin growth factor-1 and sonic hedgehog signaling pathways (101, 102), Vlahos et al. (82) prepared a prevascularization platform based on a silicone tube coated with MAA and isodecyl acrylate. After a 14-day prevascularization period in the subcutaneous space, the coated tube was removed and replaced with a marginal mass of islets suspended in collagen (250 IEQ/20 μL of collagen in mice and 4,000 IEQ/110 μL of collagen in rats). The average vessel density of the MAA-coated group exhibited a twofold increase compared with that of the unmodified subcutaneous group (105 vessels/mm^2 versus 54 vessels/mm^2). Therapeutic efficacy was observed in both SCID/beige mice and rats. However, rat studies required immunosuppression, and graft destabilization occurred by day 70 as rats grew out of their grafts.

As described above, the length of time for prevascularization often depends on the inclusion of biological factors. With biological factors, adequate prevascularization can take only 1 week (98), while without biological factors, the transient period can take at least 3 weeks (27, 82, 99). Intriguingly, Komatsu et al. (103) circumvented this nonbiologic delay and achieved 1-week adequate vascularization by combining intermittent normobaric oxygen inhalation with conventional prevascularization. The advantages of prevascularization could outweigh the disadvantages of multiple, necessary surgical procedures, and these strategies should be validated in larger animal models.

4.2.5. Cotransplantation of supporting cells. An alternative strategy for graft revascularization is the cotransplantation of islets with key proangiogenic accessory cells such as MSCs, fibroblasts, or ECs. While these approaches have their benefits, as discussed below, techniques that employ proangiogenic supporting cells must consider interactions with insulin-producing cells, as both groups must compete for nutrients and oxygen, especially at early time points (104). Also, additional technical and regulatory burdens are present when multiple cell types exist in a graft.

MSCs are multipotent progenitor cells that confer the dual functions of proangiogenic and immunomodulatory properties. They secrete trophic factors that are proangiogenic (e.g., VEGF and TGF-β) and immunomodulatory (e.g., IL-10, IL-6, and indoleamine 2,3-dioxygenase). This multidimensionality has recently been leveraged in an islet xenograft that delivered neonatal porcine islets to diabetic $Rag^{-/-}$ mice (105).

Fibroblasts are another key cell population that secrete proangiogenic factors (VEGF, FGF, PDGF) and provide structural support to blood vessels via the production of ECM molecules. The inclusion of autologous dermal fibroblasts in rat islet-delivering, autologous plasma scaffolds correlated to overexpression of proangiogenic cytokines (IL-6, IL1-β, and chitinase-3) (106). Such gene overexpression was associated with reduced apoptosis and enhanced viability of rat islets in immunodeficient mice.

ECs are strong candidates for BCRTs, given that native β cells typically neighbor ECs, and ECs deposit components of the vascular basement membrane, assisting in β cell proliferation and survival (107). Vlahos et al. (108) developed endothelialized collagen pseudoislets. Islets were deaggregated to form single-cell suspensions and embedded into submillimeter type I collagen rods coated with ECs. A total of 750 pseudoislet modules (750 modules ≈ 750 IEQ) were then subcutaneously injected in diabetic SCID/beige mice. Compared with modules loaded with regular islets, pseudoislet modules assisted faster returns to normoglycemia (10 versus 16 days) and demonstrated comparable therapeutic effects, as measured by intraperitoneal glucose tolerance tests. Likely due to the even distribution of ECs, the average distance of an $insulin^{+}$ β cell from a perfusable vessel was 5 μm, similar to lengths in the native pancreas.

4.3. Reduction of Hypoxia

Oxygen supplementation in macroscale BCRTs has both immediate and long-term benefits. During the immediate, posttransplant period that precedes adequate graft neovascularization (~7–14 days), oxygen supplementation can enhance oxygen tension at the graft site, thereby mitigating hypoxia-induced stress on insulin-producing cells (30, 109). In the long-lasting maintenance of graft survival, oxygen supplementation can overcome the mass transfer limitations imposed by immunoprotective membranes and the inevitable formation of fibrotic capsules (110). Furthermore, an increased supply of oxygen can inform the design of BCRTs. The number of therapeutic cells could be reduced, as increased oxygen levels have been shown to minimize islet death (109). Also, researchers could reduce the dimensions of macroscale BCRTs and increase loading densities, as oxygen supplementation could reduce competition for oxygen among insulin-producing cells

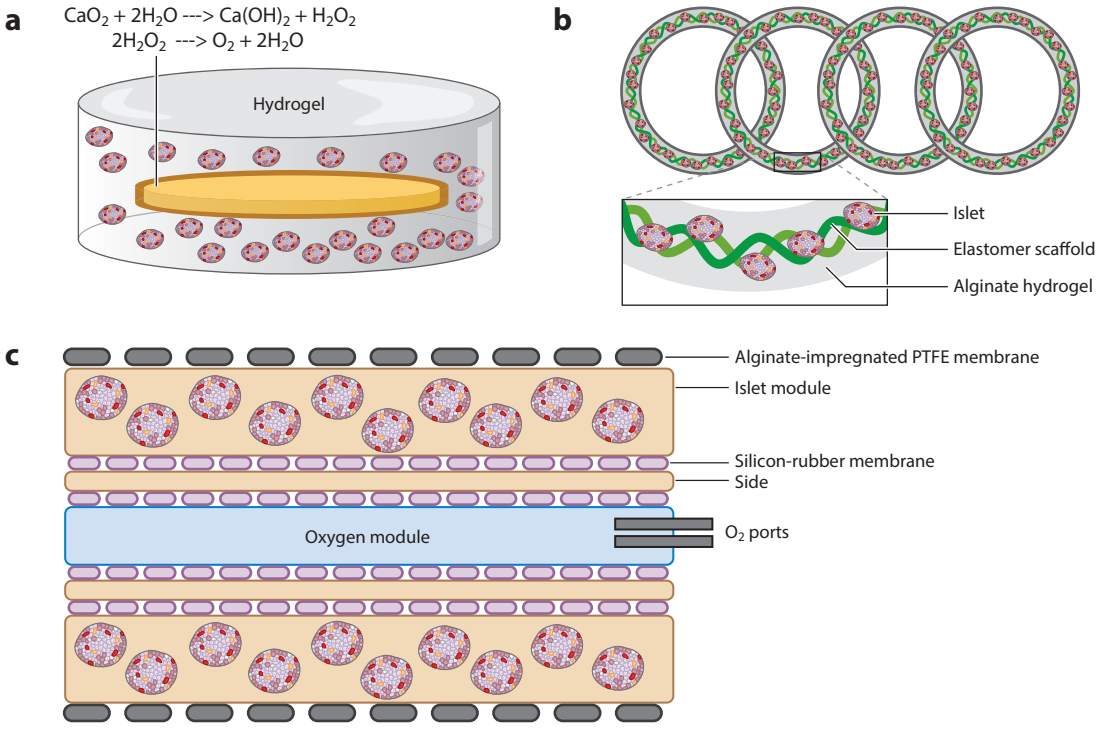

Figure 6

Approaches for the reduction of hypoxia. (*a*) In situ oxygenation strategies, such as OxySite (111), based on calcium peroxide. Panel *a* adapted with permission from Reference 111. (*b*) Improvements to the surface-area-to-volume ratio of BCRT geometries, including enhancements in mechanical robustness to fiber geometries. Interconnected toroidal hydrogels minimize the diffusional distance between islets and available oxygen. Panel *b* adapted with permission from Reference 121. (*c*) Exogenous oxygen chambers, such as the βAir macroencapsulation device. Panel *c* adapted with permission from References 1 and 16. Abbreviations: BCRT, β cell replacement therapy; PTFE, polytetrafluoroethylene.

(111). In this section, we discuss several key initiatives to enhance the oxygenation of macroscale BCRTs (**Figure 6**) (for a more comprehensive review, see 2).

4.3.1. In situ oxygen generation. In situ oxygen generators can be cotransplanted with islets in the 3D microenvironments of macroscale BCRTs. Perfluorocarbons (PFCs)—chemically stable, highly fluorinated compounds—exhibit low intermolecular cohesion forces that lend themselves to high physical solubility of gases such as oxygen (112). Indeed, due to this high physical solubility of gases, O_2 diffusion rates are 2.5-fold higher in PFCs than in culture medium (113). Emulsified perfluorodecalin added to fibrin scaffolds correlated to increased glucose stimulation indices 24 h after isolation and decreased islet levels of HIF-1α (114). Of note, however, PFCs do not generate new oxygen but, rather, only improve oxygen solubility in the transplant environment. Thus, the efficacy of delivered PFCs depends on the established oxygen levels of a transplant site.

Understanding this limitation, the Ma group (115) exploited atmospheric air as a virtually unlimited supply of oxygen. Their biphasic BCRT comprised (*a*) a subcutaneous-facing alginate hydrogel that housed islets and (*b*) a PFC oil-infused film directly exposed to atmospheric air. Atmospheric oxygen tensions measure ~160 mm Hg, more than four times that of the unmodified subcutaneous space (8–35 mm Hg). When loaded with 500 rat IEQ, the transcutaneous biphasic

device reversed hyperglycemia (blood glucose <200 mg/dL) within 1 day and maintained such for 15 days in immunocompetent, chemically induced diabetic mice.

Alternatively, calcium peroxide can be leveraged to supply oxygen to grafts, as it reacts with water to produce oxygen. The Stabler group (111) has developed OxySite, a hydrolytically reactive oxygen-generating system based on PDMS-encapsulated solid calcium peroxide. The OxySite disc was coencapsulated with islets in an agarose hydrogel. For a mouse-scale agarose macroencapsulation construct (10-mm diameter × 4-mm height), OxySite discs (5-mm diameter × 1-mm height) tripled oxygen concentrations compared with standard oxygen controls (0.39 mM versus 0.11 mM). This supplemental oxygen helped maintain the human islets' viability and function in mouse-scale constructs under hypoxic conditions, demonstrating that OxySite could support elevated loading densities of therapeutic cells. Specifically, for 48 h under 0.01 mM O_2, 1,500 human IEQ in each OxySite macroencapsulation device demonstrated a 1.6-fold increase in overall metabolic activity and glucose stimulation indices 3.7-fold higher than islets in control constructs. A key advantage of OxySite is its scalability: While this study focused on mouse- and rat-scale devices, researchers estimated that human-scale devices would only measure 12.5 cm in diameter, assuming a loading density of ∼250,000 IEQ.

4.3.2. Improvements in surface-area-to-volume ratio and fiber robustness. Enhancing surface-area-to-volume ratios of macroscale BCRT geometries can improve oxygen delivery to insulin-producing cells. Microencapsulation technologies often are more equipped to preserve islet health than their macroencapsulation counterparts due to higher surface-area-to-volume ratios, as confirmed by recent mathematical modeling (116). Traditional macroscale, immunoprotective constructs have disc (111, 117), sheet (118), or fiber (119) geometries. Current material fabrication technologies enable the development of precise yet mechanically durable geometries, decreasing diffusion distances between islets and available oxygen.

Though favorable in terms of mass transfer, traditional fiber-based geometries typically lack the mechanical robustness necessary for macroscale applications. Promisingly, the Ma group has engineered two distinct, resilient geometries that still afford the facile mass transport offered by fiber geometries: the nanofiber-enabled encapsulation device (NEED) (14) and the thread-reinforced alginate fiber for islets encapsulation (TRAFFIC) device (120). For NEEDs, An et al. (14) electrospun nylon nanofibers and collectively shaped them into macroscopically sized tubes or sheets. For tubular NEEDs, an islet-laden Matrigel hydrogel was loaded into the lumen while an alginate hydrogel was used to coat the nanofibrous shell; device dimensions for murine models were 1 inch long and 800–1,000 μm in diameter. Intraperitoneal implantation of 500 rat IEQ in NEEDs facilitated 8 weeks of diabetes correction (blood glucose <200 mg/dL) until graft retrieval in immunocompetent, diabetic mice. Also successful in xenograft feasibility, the TRAFFIC device included a centralized Ca^{2+}-releasing thread, which mechanically reinforced the islet-laden alginate hydrogel coating (120). The TRAFFIC device's overall diameter thickness was 1.3 mm, so encapsulated islets were near the surface and able to receive available oxygen in the peritoneal cavity. The sustained reversal of hyperglycemia was observed in immunocompetent, chemically diabetic mice receiving rat islets for 3 months and in immunodeficient SCID/beige mice receiving human islets for 4 months. Moreover, to demonstrate clinical translation, An et al. demonstrated the retrievability and scalability of the TRAFFIC device in dogs.

Building upon typical fiber-based geometries, the Ma group (121) has also developed interconnected toroidal hydrogels: donut-shaped, elastomer-reinforced alginate hydrogels weaved together in a chain pattern. With more surface area than spherical constructs of the same volume, these interconnected toroidal hydrogels accommodated facile mass transfer and demonstrated 12-week diabetes correction in mice. Furthermore, better than islet-loaded microcapsules, this

platform's reversible deformation permitted uncomplicated yet complete graft retrieval in the intraperitoneal cavity.

Other geometries for macroscale BCRTs have been explored to enhance oxygen availability to insulin-producing cells. In one macroencapsulation device, islets were individually seeded in microwells to prevent islet aggregation that would have obstructed individual oxygen consumption (122). In a different BCRT, the incorporation of triple hydrogen-bonding clusters in *p*(APMA-*co*-THMA) hydrogel matrices led to highly adhesive hydrogels (123), or tissue bandages. The combination of the adhesive hydrogel and an islet-encapsulating alginate hydrogel (together forming a bilayered hydrogel sheet of 1.5-mm thickness) facilitated 1 month of diabetes correction (blood glucose readings <200 mg/dL) in chemically induced diabetic mice receiving rat islets, upon stable adhesion of the platform along the body wall of the peritoneal cavity. Additionally, ex vivo glucose-stimulated insulin secretion and immunohistochemistry upon graft retrieval confirmed glucose responsiveness and normal morphology after 1 month.

4.3.3. Exogenous oxygen systems. To achieve theoretically infinite windows of oxygen production, researchers have employed exogenous oxygen systems such as chambers. Arguably the most effective oxygen-delivery system currently engineered for BCRTs, the βAir macroencapsulation device features subdermal access ports to refill oxygen (16). Daily oxygen tensions in the βAir device are replenished to supraphysiological levels; in the rat-tested device, the partial pressure of oxygen ranged from 198 to 304 mm Hg, a considerable increase from the typical 100 mm Hg of arterial blood (110). The supraphysiological levels of oxygen allow for high islet packing densities in compact devices (117). Favorable therapeutic efficacy outcomes in both rodents and nonhuman primates have led to clinical trials (discussed in the section titled Clinical Trials and Future Directions) (16, 110). Daily replenishment of exogenous oxygen requires high patient compliance. Still, perhaps the advantages of having a permanent oxygen supply in immunoprotective devices will overcome the drawbacks of absent intraislet vascularization. Another system, based on modified TheraCyte membranes and wearable electrochemical oxygen generators, may potentially generate and supply oxygen, thereby requiring less patient compliance than the βAir device (2).

From the Beta-O_2 clinical trial, as evidenced by minute, transient levels of circulating C-peptide, supplemental oxygen may not be enough to facilitate full metabolic efficacy (i.e., physiological insulin secretion and glucose response). However, supplemental oxygen maintains islet viability (NCT02064309) (77). Future work may focus on exploiting the biomaterial strategies highlighted in this review to improve insulin and glucose kinetic profiles.

4.4. Recapitulation of the Native Islet Niche

The prevention of anoikis is crucial to the engraftment of BCRTs. In this section, we review methods employed to recapitulate the native islet niche (**Figure 7**).

4.4.1. Decellularized extracellular matrix scaffolds. For restoration of the native ECM, decellularized tissues have been extensively used for artificial tissue and organ replacement. Numerous decellularization protocols exist and, in general, include enzymatic, chemical, and/or mechanical processes to isolate the ECM from cells. The ideal result is a naturally sourced scaffold that has retained its ECM structural and chemical integrity without any cellular components, thus significantly reducing immunogenicity. One choice for BCRT is decellularized pancreata. Considerable in vitro work analyzing animal decellularized pancreata has been published (124). Compared with those of animals, decellularizing pancreata from humans is more difficult due to

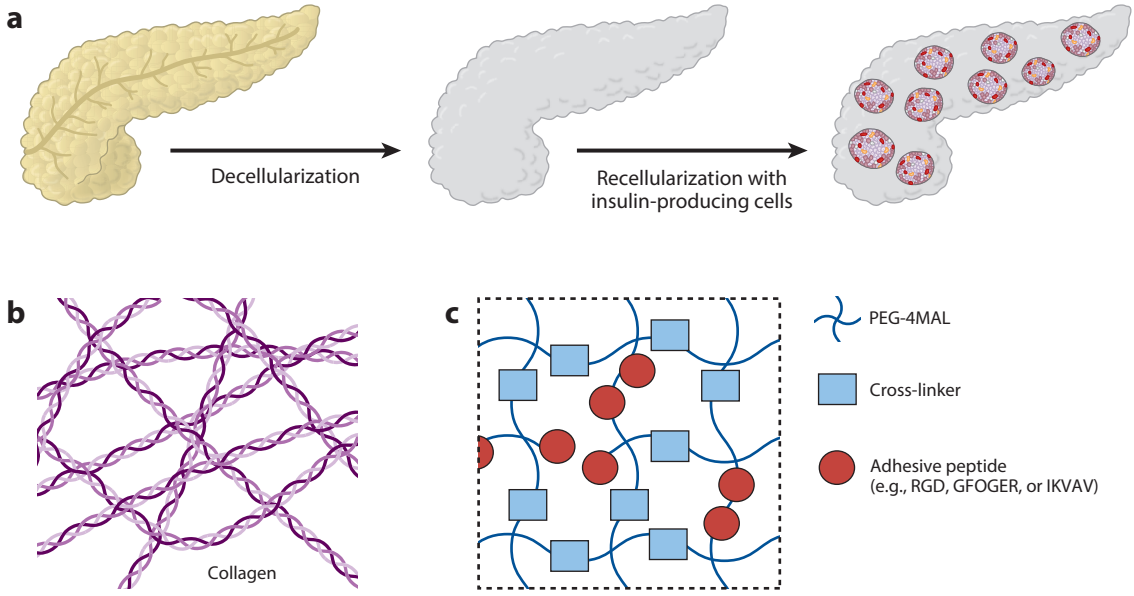

Figure 7

Approaches for recapitulation of the native islet niche. (*a*) Decellularized tissues. (*b*) ECM-based materials, such as collagen. (*c*) Artificial replacement of ECM components, such as the tethering of adhesive peptides. Abbreviations: ECM, extracellular matrix; GFOGER, glycine-phenylalanine-hydroxyproline-glycine-glutamate-arginine; IKVAV, isoleucine-lysine-valine-alanine-valine; PEG-4MAL, maleimide-terminated four-arm polyethylene glycol; RGD, arginine-glycine-aspartate.

higher lipid content; however, including homogenization during decellularization has improved lipid removal and gelation capability under physiological temperatures (125).

In the context of BCRT, minimal in vivo data exist for decellularized pancreatic scaffolds. There are results for other organs, namely, the lung (126) and pericardium (127). Citro et al.'s (126) decellularized lung scaffold preserved laminin V and collagen IV, two crucial ECM components specific to both the lung and the islet-specific pancreatic ECM. Subcutaneously implanted decellularized lung scaffolds, each loaded with 500 IEQ, maintained normoglycemia (<250 mg/dL) for 30 days in 5 of 13 NOD scid gamma mice recipients. On the other hand, all animals that received islets in a prevascularized, DL subcutaneous site or in an unmodified subcutaneous site required supplemental insulin pellets to maintain normoglycemia. Wang et al. (127) combined decellularized porcine pericardium and porcine collagen to create a bilaminated scaffold. Both layers provided ECM cues, and, separately, (*a*) the collagen was macroporous and degradable to promote vascular integration, and (*b*) the pericardium afforded mechanical, elastic stability. When loaded with 500 syngeneic IEQ and transplanted in the FP, this platform facilitated hyperglycemia reversal within 9 days and maintained hyperglycemia reversal in 83% of recipients over 300 days. (Hyperglycemia was defined as a nonfasting blood glucose level of >16.7 mM on 2 consecutive days.) Notably, with 250 IEQ, the pericardium platform achieved hyperglycemia reversal within 20 days, faster than the 38 days previously reported with fibrin gels.

4.4.2. Extracellular matrix–based materials. Another strategy to recapitulate the ECM is to employ materials based on a single ECM component. Collagen is a rational choice for macroscale BCRTs, given that several collagen structural types (I–VI) have been identified in the peripheral ECM of mature human islets (128). One recent study focused on the subcutaneous

transplantation of islets admixed with a type I human collagen mixture, referred to as an islet viability matrix (IVM) (79). Xenogeneic porcine islets admixed with IVM into immunocompetent mice demonstrated preserved function even at 51 days posttransplantation under systemic immunosuppression. Analysis of human islets in several immunodeficient murine models revealed high, early gene expressions of glucose receptor SLC2A2 and insulin, correlating with glucose homeostasis achieved within 6 h postengraftment. Intriguingly, in a diabetic cynomolgus monkey, subcutaneous transplantation of autologous islets loaded into IVM led to the preservation of islet morphology even at 918 days and insulin independence up to posttransplantation day 820. Researchers proposed that the success of IVM was mediated by the GLP1R signaling pathway, known to upregulate the antiapoptotic Bcl-2.

Fibrin matrices have also been employed in macroscale BCRTs. Fibrin is the byproduct of fibrinogen and thrombin in blood clotting; also, to promote islet survival, each fibrin monomer presents two pairs of arginine-glycine-aspartate (RGD) ligands as integrin recognition sites to promote islet survival (129). Importantly, fibrin matrix products have US Food and Drug Administration approval in various settings, reducing the regulatory burden for this application. Preclinical studies have demonstrated fibrin's proangiogenic properties on islet function and that islet function is modulated by fibrinogen-thrombin ratios (130). In a preclinical study, islets were mixed with recipient-derived, fibrinogen-rich blood plasma; after deposition of the islet-plasma slurry onto the omentum's surface, recombinant human thrombin was then added to activate gelation (131). Enhanced metabolic function and reduction of islet inflammatory biomarkers in serum were demonstrated across islet immunogenicity (syngeneic and allogeneic), diabetic animal models (rat and nonhuman primate), and islet purity (endocrine:exocrine ratios of 30:70% to >95% endocrine). In an active phase I/II clinical trial (NCT02213003), the fibrin BCRT was tested on a 43-year-old woman with a 25-year history of T1D (76). A total of 602,395 allogeneic IEQ (from one donor) were laparoscopically delivered to the omentum with patient-derived, fibrinogen-rich blood plasma. Then, the islets and blood plasma were gelled together upon thrombin application. While systemic immunosuppression was required, insulin independence was achieved at 17 days posttransplantation and maintained for 1 year. Graft decline was attributed to the switch from tacrolimus to sirolimus for immunosuppression.

4.4.3. Artificial replacement of extracellular matrix components. As an alternative to decellularized ECM materials and ECM-based materials for BCRTs, researchers have, instead, artificially recapitulated the ECM. Artificial replacement of the ECM includes coating biomaterials with ECM proteins (132), recapitulating the ECM fibrous topography with electrospun nanofibers (124), delivering GFs or supporting cells that deposit an ECM (133, 134), and tethering adhesive ligands to biomaterials (90). Tethering adhesive ligands is typically associated with synthetic biomaterials. However, some biologically derived materials (e.g., agarose from seaweed and alginate from algae) also benefit from the controlled presentation of ECM molecules. For example, ECM peptide functionalization of alginate preserved porcine islet function in vitro compared with unfunctionalized alginate (135). In a separate study with alginate microcapsules, peptide type (laminin-, fibronectin-, and collagen-derived peptides), peptide concentration (0.01–1.0 mM), and select combinations thereof modulated human islet viability and function in vitro (136).

For ECM-mimicking cues from trophic factors and supporting cells, the Shea group (133) has employed PLG microporous scaffolds to deliver trophic factors such as exendin-4 (Ex4) and insulin-like growth factor-1 (IGF-1). Ex4 is known for glucose-dependent insulin secretion and protection from apoptosis; IGF-1 decreases apoptosis, maintaining β cell mass. Fibronectin expression from BCRT sheets, developed from human dermal fibroblasts, enhanced the in vitro IL-6 expression and insulin secretory response of seeded human islets (134).

In pursuit of a reproducible platform for BCRT, minimizing the presentation of ECM components to a single component (or a select few) may be desired. Nevertheless, the selection of effective ECM protein or peptide motifs to present on biomaterial platforms remains controversial. In one study, Salvay et al. (132) found that collagen IV–coated microporous scaffolds outperformed fibronectin- and laminin-coated counterparts, as measured by the most vigorous insulin response to glucose bolus and the lowest number of days required to reverse hyperglycemia. However, another study found the high collagen IV concentrations (>100 μg/mL) did not impact islet insulin secretion in alginate microcapsules (136). Furthermore, Medina et al. (135) showed that porcine islets encapsulated in alginate microcapsules most benefitted from RGD (fibronectin) versus collagen- and laminin-derived peptides. The presentation of ECM components in various platforms and biomaterials (coencapsulation versus tethered presentation, and microscale versus macroscale) must be considered when comparing different studies.

5. CLINICAL TRIALS AND FUTURE DIRECTIONS

Tables 2 and **3** summarize completed and ongoing clinical trials of macroscale BCRTs. Such clinical trials range from closed systems without systemic immunosuppression (**Table 2**) to open systems with systemic immunosuppression (**Table 3**). The ultimate goal of BCRTs is the restoration of tight, autonomous glucose control—physiologically efficient glucose metabolism. Long-term insulin independence remains the holy grail of BCRTs, although it is still unattainable in most clinical trials (13). Nevertheless, we remain optimistic, as BCRTs continue to demonstrate graft survival and only minor surgical complications as well as reductions in severe hypoglycemic episodes and exogenous insulin requirements—stepping stones of progress.

The synthesis of highlighted research suggests that the design of an effective macroscale BCRT must employ a multipronged approach. However, efforts to address one major challenge often conflict with those for another. Both open and closed systems have advantages and disadvantages. Perforated, cell-permissive membranes allow vascular integration yet require immunosuppression. Encapsulation approaches can eliminate the need for immunosuppression yet present

Table 2 Clinical trials of macroscale closed systems

Platform	Overview	Clinical trial
ViaCyte PEC-Encap (VC-01)	Macroencapsulation of hESC-derived pancreatic progenitors, allowed to mature in vivo Subcutaneous implantation Two, four, or six implants per recipient No immunosuppression	I/II NCT02239354 NCT02939118
ViaCyte VC01–103	Macroencapsulation of hESC-derived pancreatic progenitors utilizing Gore proprietary material Subcutaneous implantation Monitored for up to 12 months for safety, tolerability, and efficacy No immunosuppression	I/II NCT04678557
Beta-O_2's βAir device	Macroencapsulation of allogeneic islets paired with exogenous oxygen chamber for daily refilling of oxygen Subcutaneous implantation One to two devices for each patient 155,000–180,000 IEQ (1,800–4,600 IEQ/kg) for each device No immunosuppression	I/II NCT02064309

Abbreviations: hESC, human embryonic stem cell; IEQ, islet equivalent.

Table 3 Clinical trials of macroscale open systems

Platform	Overview	Clinical trial
Sernova Cell Pouch	Cell pouch loaded with allogeneic islets following prevascularization Subcutaneous implantation ≥6 weeks of prevascularization >3,000 IEQ/kg for each device Requires immunosuppression	I/II NCT03513939
Plasma-thrombin gel	Gel formed with recombinant thrombin and patient-derived plasma, then loaded with allogeneic islets Laparoscopic delivery to the omentum ≥5,000 IEQ/kg Requires immunosuppression	I/II NCT02213003
ViaCyte PEC-Direct (VC-02)	Perforated membrane (to allow for vascular integration) loaded with hESC-derived pancreatic progenitors Subcutaneous implantation Requires immunosuppression	I/II NCT03163511 NCT03162926

Abbreviations: hESC, human embryonic stem cell; IEQ, islet equivalent.

mass transfer limitations for nutrients and oxygen. Some notable combinatory approaches include (*a*) the dual-reservoir encapsulation system that integrates in situ prevascularization and local immunosuppressant delivery (137) and (*b*) the combination of a proangiogenic scaffold with oxygen-generating microparticles (109).

Moving forward, efforts to develop biomaterial-based, macroscale BCRT platforms must converge with efforts to optimize alternative cell sources. One fixed type of macroscale BCRT will not accommodate all types of grafts. Subtypes of porcine islets and SC-β cells require different transplant conditions. For example, neonatal, juvenile, and mature porcine islets differ in oxygen demand and transcriptomes (138); specifically, neonatal islets are more resistant to hypoxia-induced apoptosis than their mature counterparts (139). Tunability in biomaterial design will assist in accommodating various cell types.

New technologies and chemistries will inform the design of future macroscale BCRTs. Three-dimensional printing and bioprinting have already begun to revolutionize BCRTs, as they could be used to print high-resolution vessel networks near islets (140). As previously discussed, zwitterionic polymers are promising materials to mitigate FBR against implants. Novel technologies may allow researchers to noninvasively monitor oxygen and the formation of vasculature; they include luciferase signaling (141) and photoacoustic imaging of angiogenesis (142).

Additionally, research conducted at the nano- and microscales can be applied to macroscale BCRTs. Microphysiological systems and organ-on-a-chip platforms, such as those based on alginate microcapsules (143) and decellularized tissue (144), may provide insights into necessary ECM cues and biomaterial properties and could be used to screen both pharmaceuticals or biomaterials in time periods shorter than complete transplantation studies.

In pursuit of translation to humans, biomaterial design for macroscale BCRTs should include desirable clinical traits. Retrievability and, consequently, safety are critical attributes for macroscale BCRTs. Still, other features are merited, including material choice that will accelerate regulatory approval (e.g., synthetic biomaterials, alginate, and fibrin), reproducibility of constructs (achievable through synthetic biomaterials and/or advanced fabrication technologies), scalability from rodent to large animal models (16, 82, 111, 120, 121), and ease in the replenishment of therapeutic cells (115, 145). Advances in biomaterial research and technologies have paved the way for functional macroscale BCRTs. The ideal macroscale BCRT should limit early

posttransplant inflammation and FBR; be specific in manipulating the host immune response; and provide a hospitable transplant microenvironment with robust vascularization, adequate oxygen, and necessary ECM cues.

DISCLOSURE STATEMENT

A.J.G. is a cofounder and scientific advisory board member of iTolerance, a start-up company focused on a biomaterial-based strategy to induce immune acceptance of transplanted islets for the treatment of T1D.

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LITERATURE CITED

1. Desai T, Shea LD. 2017. Advances in islet encapsulation technologies. *Nat. Rev. Drug Discov.* 16(5):338–50
2. Papas KK, De Leon H, Suszynski TM, Johnson RC. 2019. Oxygenation strategies for encapsulated islet and beta cell transplants. *Adv. Drug Deliv. Rev.* 139:139–56
3. Mathieu C, Gillard P, Benhalima K. 2017. Insulin analogues in type 1 diabetes mellitus: getting better all the time. *Nat. Rev. Endocrinol.* 13(7):385–99
4. Shah R, Patel M, Maahs D, Shah V. 2016. Insulin delivery methods: past, present and future. *Int. J. Pharm. Investig.* 6(1):1–9
5. Latres E, Finan DA, Greenstein JL, Kowalski A, Kieffer TJ. 2019. Navigating two roads to glucose normalization in diabetes: automated insulin delivery devices and cell therapy. *Cell Metab.* 29(3):545–63
6. Foster NC, Beck RW, Miller KM, Clements MA, Rickels MR, et al. 2019. State of type 1 diabetes management and outcomes from the T1D Exchange in 2016–2018. *Diabetes Technol. Ther.* 21(2):66–72
7. Shapiro AMJ, Lakey JRT, Ryan EA, Korbutt GS, Toth E, et al. 2000. Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. *N. Engl. J. Med.* 343(4):230–38
8. Hering BJ, Clarke WR, Bridges ND, Eggerman TL, Alejandro R, et al. 2016. Phase 3 trial of transplantation of human islets in type 1 diabetes complicated by severe hypoglycemia. *Diabetes Care* 39(7):1230–40
9. Shapiro AMJ, Pokrywczynska M, Ricordi C. 2017. Clinical pancreatic islet transplantation. *Nat. Rev. Endocrinol.* 13(5):268–77
10. Ludwig B. 2019. Islet immunoisolation by macroencapsulation. In *Transplantation, Bioengineering, and Regeneration of the Endocrine Pancreas*, Vol. 1, ed. G Orlando, L Piemonti, C Ricordi, RJ Stratta, RWG Gruessner, pp. 751–59. London: Elsevier
11. Weaver JD, Headen DM, Aquart J, Johnson CT, Shea LD, et al. 2017. Vasculogenic hydrogel enhances islet survival, engraftment, and function in leading extrahepatic sites. *Sci. Adv.* 3(6):e1700184
12. Ernst AU, Bowers DT, Wang L-H, Shariati K, Plesser MD, et al. 2019. Nanotechnology in cell replacement therapies for type 1 diabetes. *Adv. Drug Deliv. Rev.* 139:116–38
13. Marfil-Garza BA, Polishevska K, Pepper AR, Korbutt GS. 2020. Current state and evidence of cellular encapsulation strategies in type 1 diabetes. *Compr. Physiol.* 10(3):839–78
14. An D, Ji Y, Chiu A, Lu YC, Song W, et al. 2015. Developing robust, hydrogel-based, nanofiber-enabled encapsulation devices (NEEDs) for cell therapies. *Biomaterials* 37:40–48
15. Morihara K, Oka T, Tsuzuki H. 1979. Semi-synthesis of human insulin by trypsin-catalysed replacement. *Nature* 280(2):412–13
16. Ludwig B, Ludwig S, Steffen A, Knauf Y, Zimerman B, et al. 2017. Favorable outcome of experimental islet xenotransplantation without immunosuppression in a nonhuman primate model of diabetes. *PNAS* 114(44):11745–50

17. Rezania A, Bruin JE, Arora P, Rubin A, Batushansky I, et al. 2014. Reversal of diabetes with insulin-producing cells derived in vitro from human pluripotent stem cells. *Nat. Biotechnol.* 32(11):1121–33
18. Scobie L, Denner J, Schuurman HJ. 2017. Inactivation of porcine endogenous retrovirus in pigs using CRISPR-Cas9, editorial commentary. *Xenotransplantation* 24(6):1303–7
19. Kroon E, Martinson LA, Kadoya K, Bang AG, Kelly OG, et al. 2008. Pancreatic endoderm derived from human embryonic stem cells generates glucose-responsive insulin-secreting cells in vivo. *Nat. Biotechnol.* 26(4):443–52
20. Rorsman P, Ashcroft FM. 2018. Pancreatic β-cell electrical activity and insulin secretion: of mice and men. *Physiol. Rev.* 98(1):117–214
21. Carlsson PO, Palm F, Andersson A, Liss P. 2001. Markedly decreased oxygen tension in transplanted rat pancreatic islets irrespective of the implantation site. *Diabetes* 50(3):489–95
22. Pedraza E, Coronel MM, Fraker CA, Ricordi C, Stabler CL. 2012. Preventing hypoxia-induced cell death in beta cells and islets via hydrolytically activated, oxygen-generating biomaterials. *PNAS* 109(11):4245–50
23. Schuit F, De Vos A, Farfari S, Moens K, Pipeleers D, et al. 1997. Metabolic fate of glucose in purified islet cells. Glucose-regulated anaplerosis in β cells. *J. Biol. Chem.* 272(30):18572–79
24. Dionne KE, Colton CK, Yarmush ML. 1993. Effect of hypoxia on insulin secretion by isolated rat and canine islets of Langerhans. *Diabetes* 42(1):12–21
25. Child A, Larkin EJ, Fontaine MJ. 2020. Co-encapsulation of ECM proteins to enhance pancreatic islet cell function. In *Transplantation, Bioengineering, and Regeneration of the Endocrine Pancreas*, Vol. 2, ed. G Orlando, L Piemonti, C Ricordi, RJ Stratta, RWG Gruessner, pp. 307–13. London: Elsevier
26. Bosco D, Meda P, Halban PA, Rouiller DG. 2000. Importance of cell-matrix interactions in rat islet β-cell secretion in vitro: role of α6β1 integrin. *Diabetes* 49(2):233–43
27. Pepper AR, Gala-Lopez B, Pawlick R, Merani S, Kin T, Shapiro AMJ. 2015. A prevascularized subcutaneous device-less site for islet and cellular transplantation. *Nat. Biotechnol.* 33:518–23
28. Bowers DT, Song W, Wang L-H, Ma M. 2019. Engineering the vasculature for islet transplantation. *Acta Biomater*. 95:131–51
29. Gan WJ, Do OH, Cottle L, Ma W, Kosobrodova E, et al. 2018. Local integrin activation in pancreatic β cells targets insulin secretion to the vasculature. *Cell Rep*. 24(11):2819–26.e3
30. Jones GL, Juszczak MT, Hughes SJ, Kooner P, Powis SH, Press M. 2007. Time course and quantification of pancreatic islet revascularizton following intraportal transplantation. *Cell Transplant*. 16(5):505–16
31. Paredes-Juarez GA, Sahasrabudhe NM, Tjoelker RS, De Haan BJ, Engelse MA, et al. 2015. DAMP production by human islets under low oxygen and nutrients in the presence or absence of an immunoisolating-capsule and necrostatin-1. *Sci. Rep.* 5:14623
32. Ko SH, Ryu GR, Kim S, Ahn YB, Yoon KH, et al. 2008. Inducible nitric oxide synthase-nitric oxide plays an important role in acute and severe hypoxic injury to pancreatic beta cells. *Transplantation* 85(3):323–30
33. Marzorati S, Melzi R, Nano R, Antonioli B, Di Carlo V, et al. 2004. In vitro modulation of monocyte chemoattractant protein-1 release in human pancreatic islets. *Transplant. Proc.* 36(3):607–8
34. Moritz W, Meier F, Stroka DM, Giuliani M, Kugelmeier P, et al. 2002. Apoptosis in hypoxic human pancreatic islets correlates with HIF-1α expression. *FASEB J.* 16(7):745–47
35. Tjernberg J, Ekdahl KN, Lambris JD, Korsgren O, Nilsson B. 2008. Acute antibody-mediated complement activation mediates lysis of pancreatic islets cells and may cause tissue loss in clinical islet transplantation. *Transplantation* 85(8):1193–99
36. Biarnés M, Montolio M, Nacher V, Raurell M, Soler J, Montanya E. 2002. β-Cell death and mass in syngeneically transplanted islets exposed to short- and long-term hyperglycemia. *Diabetes* 51(1):66–72
37. Paty BW, Harmon JS, Marsh CL, Robertson RP. 2002. Inhibitory effects of immunosuppressive drugs on insulin secretion from HIT-T15 cells and Wistar rat islets. *Transplantation* 73(3):353–57
38. Scharp DW, Marchetti P. 2014. Encapsulated islets for diabetes therapy: history, current progress, and critical issues requiring solution. *Adv. Drug Deliv. Rev.* 67–68:35–73
39. Sullivan SJ, Maki T, Borland KM, Mahoney MD, Solomon BA, et al. 1991. Biohybrid artificial pancreas: long-term implantation studies in diabetic, pancreatectomized dogs. *Science* 252(5006):718–21

40. Song S, Blaha C, Moses W, Park J, Wright N, et al. 2017. An intravascular bioartificial pancreas device (iBAP) with silicon nanopore membranes (SNM) for islet encapsulation under convective mass transport. *Lab Chip* 17(10):1778–92
41. Pedraza E, Brady AC, Fraker CA, Molano RD, Sukert S, et al. 2013. Macroporous three-dimensional PDMS scaffolds for extrahepatic islet transplantation. *Cell Transplant*. 22(7):1123–25
42. Coronel MM, Stabler CL. 2013. Engineering a local microenvironment for pancreatic islet replacement. *Curr. Opin. Biotechnol.* 24(5):900–8
43. Kharbikar BN, Chendke GS, Desai TA. 2021. Modulating the foreign body response of implants for diabetes treatment. *Adv. Drug Deliv. Rev.* 174:87–113
44. Fuchs S, Ernst AU, Wang L-H, Shariati K, Wang X, et al. 2021. Hydrogels in emerging technologies for type 1 diabetes. *Chem. Rev.* 121(18):11458–526
45. Zhang L, Cao Z, Bai T, Carr L, Ella-Menye JR, et al. 2013. Zwitterionic hydrogels implanted in mice resist the foreign-body reaction. *Nat. Biotechnol.* 31(6):553–56
46. Dusseault J, Tam SK, Ménard M, Polizu S, Jourdan G, et al. 2006. Evaluation of alginate purification methods: effect on polyphenol, endotoxin, and protein contamination. *J. Biomed. Mater. Res. A* 76(2):243–51
47. Martinsen A, Skjåk-Bræk G, Smidsrød O. 1989. Alginate as immobilization material: I. Correlation between chemical and physical properties of alginate gel beads. *Biotechnol. Bioeng.* 33(1):79–89
48. Veiseh O, Doloff JC, Ma M, Vegas AJ, Tam HH, et al. 2015. Size- and shape-dependent foreign body immune response to materials implanted in rodents and non-human primates. *Nat. Mater.* 14(6):643–51
49. Hu S, de Vos P. 2019. Polymeric approaches to reduce tissue responses against devices applied for islet-cell encapsulation. *Front. Bioeng. Biotechnol.* 7:134
50. Phelps EA, Landázuri N, Thulé PM, Taylor WR, García AJ. 2010. Bioartificial matrices for therapeutic vascularization. *PNAS* 107(8):3323–28
51. Partini M, Pantani R. 2007. FTIR analysis of hydrolysis in aliphatic polyesters. *Polym. Degrad. Stab.* 92(8):1491–97
52. Neufeld T, Ludwig B, Barkai U, Weir GC, Colton CK, et al. 2013. The efficacy of an immunoisolating membrane system for islet xenotransplantation in minipigs. *PLOS ONE* 8(8):e70150
53. Marchioli G, Luca A Di, de Koning E, Engelse M, Van Blitterswijk CA, et al. 2016. Hybrid polycaprolactone/alginate scaffolds functionalized with VEGF to promote de novo vessel formation for the transplantation of islets of Langerhans. *Adv. Healthc. Mater.* 5(13):1606–16
54. Xie X, Doloff JC, Yesilyurt V, Sadraei A, McGarrigle JJ, et al. 2018. Reduction of measurement noise in a continuous glucose monitor by coating the sensor with a zwitterionic polymer. *Nat. Biomed. Eng.* 2(12):894–906
55. Liu Q, Chiu A, Wang LH, An D, Zhong M, et al. 2019. Zwitterionically modified alginates mitigate cellular overgrowth for cell encapsulation. *Nat. Commun.* 10:5262
56. Li B, Yuan Z, Jain P, Hung HC, He Y, et al. 2020. De novo design of functional zwitterionic biomimetic material for immunomodulation. *Sci. Adv.* 6(22):eaba0754
57. Stabler CL, Li Y, Stewart JM, Keselowsky BG. 2019. Engineering immunomodulatory biomaterials for type 1 diabetes. *Nat. Rev. Mater.* 4(6):429–50
58. Gibly RF, Graham JG, Luo X, Lowe WL, Hering BJ, Shea LD. 2011. Advancing islet transplantation: from engraftment to the immune response. *Diabetologia* 54(10):2494–505
59. Jiang K, Weaver JD, Li Y, Chen X, Liang J, Stabler CL. 2017. Local release of dexamethasone from macroporous scaffolds accelerates islet transplant engraftment by promotion of anti-inflammatory M2 macrophages. *Biomaterials* 114:71–81
60. Haque MR, Lee DY, Ahn CH, Jeong JH, Byun Y. 2014. Local co-delivery of pancreatic islets and liposomal clodronate using injectable hydrogel to prevent acute immune reactions in a type 1 diabetes. *Pharm. Res.* 31(9):2453–62
61. Thomson AW, Bonham CA, Zeevi A. 1995. Mode of action of tacrolimus (FK506): molecular and cellular mechanisms. *Ther. Drug Monit.* 17(6):584–91
62. Pathak S, Regmi S, Gupta B, Poudel BK, Pham TT, et al. 2017. Drug delivery single synchronous delivery of FK506-loaded polymeric microspheres with pancreatic islets for the successful treatment of streptozocin-induced diabetes in mice. *Drug Deliv.* 24(1):1350–59

63. Farah S, Doloff JC, Müller P, Sadraei A, Han HJ, et al. 2019. Long-term implant fibrosis prevention in rodents and non-human primates using crystallized drug formulations. *Nat. Mater.* 18(8):892–904
64. Headen DM, Woodward KB, Coronel MM, Shrestha P, Weaver JD, et al. 2018. Local immunomodulation with Fas ligand-engineered biomaterials achieves allogeneic islet graft acceptance. *Nat. Mater.* 17(8):732–39
65. Graham JG, Zhang X, Goodman A, Pothoven K, Houlihan J, et al. 2013. PLG scaffold delivered antigen-specific regulatory T cells induce systemic tolerance in autoimmune diabetes. *Tissue Eng. A* 19(11–12):1465–75
66. Coronel MM, Martin KE, Hunckler MD, Barber G, O'Neill EB, et al. 2020. Immunotherapy via PD-L1-presenting biomaterials leads to long-term islet graft survival. *Sci. Adv.* 6(35):eaba5573
67. Schweicher J. 2014. Membranes to achieve immunoprotection of transplanted islets. *Front. Biosci.* 19(1):49
68. Colton CK. 1995. Implantable biohybrid artificial organs. *Cell Transplant.* 4(4):415–36
69. Nafea EH, Marson A, Poole-Warren LA, Martens PJ. 2011. Immunoisolating semi-permeable membranes for cell encapsulation: focus on hydrogels. *J. Control. Release* 154(2):110–22
70. Li Y, Frei AW, Yang EY, Labrada-Miravet I, Sun C, et al. 2020. In vitro platform establishes antigen-specific $CD8^+$ T cell cytotoxicity to encapsulated cells via indirect antigen recognition. *Biomaterials* 256:120182
71. Barkai U, Rotem A, de Vos P. 2016. Survival of encapsulated islets: more than a membrane story. *World J. Transplant.* 6(1):69
72. Cantarelli E, Piemonti L. 2011. Alternative transplantation sites for pancreatic islet grafts. *Curr. Diab. Rep.* 11(5):364–74
73. Perez VL, Caicedo A, Berman DM, Arrieta E, Abdulreda MH, et al. 2011. The anterior chamber of the eye as a clinical transplantation site for the treatment of diabetes: a study in a baboon model of diabetes. *Diabetologia* 54(5):1121–26
74. Kakabadze Z, Shanava K, Ricordi C, Shapiro AMJ, Gupta S, Berishvili E. 2012. An isolated venous sac as a novel site for cell therapy in diabetes mellitus. *Transplantation* 94(4):319–24
75. Wszola M, Berman A, Gorski L, Ostaszewska A, Serwanska-Swietek M, et al. 2018. Endoscopic islet autotransplantation into gastric submucosa—1000-day follow-up of patients. *Transplant. Proc.* 50(7):2119–23
76. Baidal DA, Ricordi C, Berman DM, Alvarez A, Padilla N, et al. 2017. Bioengineering of an intraabdominal endocrine pancreas. *N. Engl. J. Med.* 376(19):1887–89
77. Carlsson P-O, Espes D, Sedigh A, Rotem A, Zimerman B, et al. 2018. Transplantation of macroencapsulated human islets within the bioartificial pancreas βAir to patients with type 1 diabetes mellitus. *Am. J. Transplant.* 18(7):1735–44
78. Agulnick AD, Ambruzs DM, Moorman MA, Bhoumik A, Cesario RM, et al. 2015. Insulin-producing endocrine cells differentiated in vitro from human embryonic stem cells function in macroencapsulation devices in vivo. *Stem Cells Transl. Med.* 4(10):1214–22
79. Yu M, Agarwal D, Korutla L, May CL, Wang W, et al. 2020. Islet transplantation in the subcutaneous space achieves long-term euglycaemia in preclinical models of type 1 diabetes. *Nat. Metab.* 2(10):1013–20
80. Yang B, Cao G, Cai K, Wang G, Li P, et al. 2020. VEGF-modified PVA/silicone nanofibers enhance islet function transplanted in subcutaneous site followed by device-less procedure. *Int. J. Nanomed.* 15:587–99
81. Song W, Chiu A, Wang L-H, Schwartz RE, Li B, et al. 2019. Engineering transferrable microvascular meshes for subcutaneous islet transplantation. *Nat. Commun.* 10:4602
82. Vlahos AE, Talior-Volodarsky I, Kinney SM, Sefton M V. 2020. A scalable device-less biomaterial approach for subcutaneous islet transplantation. *Biomaterials* 269:120499
83. Brauker JH, Carr-Brendel VE, Martinson LA, Crudele J, Johnston WD, Johnson RC. 1995. Neovascularization of synthetic membranes directed by membrane microarchitecture. *J. Biomed. Mater. Res.* 29(12):1517–24
84. Madden LR, Mortisen DJ, Sussman EM, Dupras SK, Fugate JA, et al. 2010. Proangiogenic scaffolds as functional templates for cardiac tissue engineering. *PNAS* 107(34):15211–16
85. Chiu YC, Cheng MH, Engel H, Kao SW, Larson JC, et al. 2011. The role of pore size on vascularization and tissue remodeling in PEG hydrogels. *Biomaterials* 32(26):6045–51

86. Choi S-W, Zhang Y, MacEwan MR, Xia Y. 2013. Neovascularization in biodegradable inverse opal scaffolds with uniform and precisely controlled pore sizes. *Adv. Healthc. Mater.* 2(1):145–54
87. Huang HH, Harrington S, Stehno-Bittel L. 2018. The flaws and future of islet volume measurements. *Cell Transplant.* 27(7):1017–26
88. Lammert E, Gu G, McLaughlin M, Brown D, Brekken R, et al. 2003. Role of VEGF-A in vascularization of pancreatic islets. *Curr. Biol.* 13(12):1070–74
89. Gebe JA, Preisinger A, Gooden MD, D'Amico LA, Vernon RB. 2018. Local, controlled release in vivo of vascular endothelial growth factor within a subcutaneous scaffolded islet implant reduces early islet necrosis and improves performance of the graft. *Cell Transplant.* 27(3):531–41
90. Weaver JD, Headen DM, Hunckler MD, Coronel MM, Stabler CL, García AJ. 2018. Design of a vascularized synthetic poly(ethylene glycol) macroencapsulation device for islet transplantation. *Biomaterials* 172:54–65
91. Brady A-C, Martino MM, Pedraza E, Sukert S, Pileggi A, et al. 2013. Proangiogenic hydrogels within macroporous scaffolds enhance islet engraftment in an extrahepatic site. *Tissue Eng. A* 19(23–24):2544–52
92. Mao D, Zhu M, Zhang X, Ma R, Yang X, et al. 2017. A macroporous heparin-releasing silk fibroin scaffold improves islet transplantation outcome by promoting islet revascularisation and survival. *Acta Biomater.* 59:210–20
93. Nguyen TT, Emami F, Yook S, Nguyen HT, Pham TT, et al. 2020. Local release of NECA (5′-(N-ethylcarboxamido)adenosine) from implantable polymeric sheets for enhanced islet revascularization in extrahepatic transplantation site. *J. Control. Release* 321:509–18
94. Bowers DT, Olingy CE, Chhabra P, Langman L, Merrill PH, et al. 2018. An engineered macroencapsulation membrane releasing FTY720 to precondition pancreatic islet transplantation. *J. Biomed. Mater. Res. B Appl. Biomater.* 106(2):555–68
95. Richardson TP, Peters MC, Ennett AB, Mooney DJ. 2001. Polymeric system for dual growth factor delivery. *Nat. Biotechnol.* 19(11):1029–34
96. Tengood JE, Kovach KM, Vescovi PE, Russell AJ, Little SR. 2010. Sequential delivery of vascular endothelial growth factor and sphingosine 1-phosphate for angiogenesis. *Biomaterials* 31(30):7805–12
97. Smink AM, Li S, Swart DH, Hertsig DT, de Haan BJ, et al. 2017. Stimulation of vascularization of a subcutaneous scaffold applicable for pancreatic islet-transplantation enhances immediate post-transplant islet graft function but not long-term normoglycemia. *J. Biomed. Mater. Res. A* 105(9):2533–42
98. Luan NM, Iwata H. 2014. Long-term allogeneic islet graft survival in prevascularized subcutaneous sites without immunosuppressive treatment. *Am. J. Transplant.* 344(7):1533–42
99. Smink AM, Hertsig DT, Schwab L, Van Apeldoorn AA, De Koning E, et al. 2017. A retrievable, efficacious polymeric scaffold for subcutaneous transplantation of rat pancreatic islets. *Ann. Surg.* 266(1):149–57
100. Pepper AR, Pawlick R, Bruni A, Wink J, Rafiei Y, et al. 2017. Transplantation of human pancreatic endoderm cells reverses diabetes post transplantation in a prevascularized subcutaneous site. *Stem Cell Rep.* 8(6):1689–700
101. Talior-Volodarsky I, Mahou R, Zhang D, Sefton M. 2017. The role of insulin growth factor-1 on the vascular regenerative effect of MAA coated disks and macrophage-endothelial cell crosstalk. *Biomaterials* 144:199–210
102. Lisovsky A, Zhang DKY, Sefton MV. 2016. Effect of methacrylic acid beads on the sonic hedgehog signaling pathway and macrophage polarization in a subcutaneous injection mouse model. *Biomaterials* 98:203–14
103. Komatsu H, Gonzalez N, Kandeel F, Mullen Y. 2020. Intermittent normobaric oxygen inhalation enhances subcutaneous prevascularization for cell transplantation. *Microvasc. Res.* 132:104070
104. Vlahos AE, Cober N, Sefton MV. 2017. Modular tissue engineering for the vascularization of subcutaneously transplanted pancreatic islets. *PNAS* 114(35):9337–42
105. Kuppan P, Seeberger K, Kelly S, Rosko M, Adesida A, et al. 2020. Co-transplantation of human adipose-derived mesenchymal stem cells with neonatal porcine islets within a prevascularized subcutaneous space augments the xenograft function. *Xenotransplantation* 27(4):e12581

106. Perez-Basterrechea M, Martinez Esteban M, Alvarez-Viejo M, Fontanil T, Cal S, et al. 2017. Fibroblasts accelerate islet revascularization and improve long-term graft survival in a mouse model of subcutaneous islet transplantation. *PLOS ONE* 12(7):e0180695
107. Townsend SE, Gannon M. 2019. Extracellular matrix-associated factors play critical roles in regulating pancreatic β-cell proliferation and survival. *Endocrinology* 160(8):1885–94
108. Vlahos AE, Kinney SM, Kingston BR, Keshavjee S, Won SY, et al. 2020. Endothelialized collagen based pseudo-islets enables tuneable subcutaneous diabetes therapy. *Biomaterials* 232:119710
109. Montazeri L, Hojjati-Emami S, Bonakdar S, Tahamtani Y, Hajizadeh-Saffar E, et al. 2016. Improvement of islet engrafts by enhanced angiogenesis and microparticle-mediated oxygenation. *Biomaterials* 89:157–65
110. Barkai U, Weir GC, Colton CK, Ludwig B, Bornstein SR, et al. 2013. Enhanced oxygen supply improves islet viability in a new bioartificial pancreas. *Cell Transplant.* 22(8):1463–76
111. Coronel MM, Liang J-P, Li Y, Stabler CL. 2019. Oxygen generating biomaterial improves the function and efficacy of beta cells within a macroencapsulation device. *Biomaterials* 210:1–11
112. Riess JG. 2006. Perfluorocarbon-based oxygen delivery. *Artif. Cells Blood Substit. Biotechnol.* 34(6):567–80
113. Krafft MP, Chittofrati A, Reiss JG. 2003. Emulsions and microemulsions with a fluorocarbon phase. *Curr. Opin. Colloid Interface Sci.* 8(3):251–58
114. Maillard E, Juszczak MT, Clark A, Hughes SJ, Gray DRW, Johnson PRV. 2011. Perfluorodecalin-enriched fibrin matrix for human islet culture. *Biomaterials* 32(35):9282–89
115. An D, Wang L-H, Ernst AU, Chiu A, Lu Y-C, et al. 2019. An atmosphere-breathing refillable biphasic device for cell replacement therapy. *Adv. Mater.* 31(52):1905135
116. Cao R, Avgoustiniatos E, Papas K, Vos P, Lakey JRT. 2020. Mathematical predictions of oxygen availability in micro- and macro-encapsulated human and porcine pancreatic islets. *J. Biomed. Mater. Res. B Appl. Biomater.* 108(2):343–52
117. Evron Y, Colton CK, Ludwig B, Weir GC, Zimermann B, et al. 2018. Long-term viability and function of transplanted islets macroencapsulated at high density are achieved by enhanced oxygen supply. *Sci. Rep.* 8:6508
118. Lamb M, Storrs R, Li S, Liang O, Laugenour K, et al. 2011. Function and viability of human islets encapsulated in alginate sheets: in vitro and in vivo culture. *Transplant. Proc.* 43(9):3265–66
119. Onoe H, Okitsu T, Itou A, Kato-Negishi M, Gojo R, et al. 2013. Metre-long cell-laden microfibres exhibit tissue morphologies and functions. *Nat. Mater.* 12(6):584–90
120. An D, Chiu A, Flanders JA, Song W, Shou D, et al. 2017. Designing a retrievable and scalable cell encapsulation device for potential treatment of type 1 diabetes. *PNAS* 115(2):E263–72
121. Ernst AU, Wang LH, Ma M. 2019. Interconnected toroidal hydrogels for islet encapsulation. *Adv. Healthc. Mater.* 8(12):1900423
122. Skrzypek K, Groot Nibbelink M, van Lente J, Buitinga M, Engelse MA, et al. 2017. Pancreatic islet macroencapsulation using microwell porous membranes. *Sci. Rep.* 7(1):9186
123. Chen J, Wang D, Wang LH, Liu W, Chiu A, et al. 2020. An adhesive hydrogel with "load-sharing" effect as tissue bandages for drug and cell delivery. *Adv. Mater.* 32(43):2001628
124. Zhu Y, Wang D, Yao X, Wang M, Zhao Y, et al. 2021. Biomimetic hybrid scaffold of electrospun silk fibroin and pancreatic decellularized extracellular matrix for islet survival. *J. Biomater. Sci. Polym. Ed.* 32(2):151–65
125. Sackett SD, Tremmel DM, Ma F, Feeney AK, Maguire RM, et al. 2018. Extracellular matrix scaffold and hydrogel derived from decellularized and delipidized human pancreas. *Sci. Rep.* 8:10452
126. Citro A, Moser PT, Dugnani E, Rajab KT, Ren X, et al. 2019. Biofabrication of a vascularized islet organ for type 1 diabetes. *Biomaterials* 199:40–51
127. Wang X, Wang K, Zhang W, Qiang M, Luo Y. 2017. A bilaminated decellularized scaffold for islet transplantation: structure, properties and functions in diabetic mice. *Biomaterials* 138:80–90
128. Llacua LA, Faas MM, de Vos P. 2018. Extracellular matrix molecules and their potential contribution to the function of transplanted pancreatic islets. *Diabetologia* 61(6):1261–72
129. Janmey PA, Winer JP, Weisel JW. 2009. Fibrin gels and their clinical and bioengineering applications. *J. R. Soc. Interface* 6(30):1–10

130. Kim JS, Lim JH, Nam HY, Lim HJ, Shin JS, et al. 2012. In situ application of hydrogel-type fibrin-islet composite optimized for rapid glycemic control by subcutaneous xenogeneic porcine islet transplantation. *J. Control. Release* 162(2):382–90
131. Berman DM, Molano RD, Fotino C, Ulissi U, Gimeno J, et al. 2016. Bioengineering the endocrine pancreas: intraomental islet transplantation within a biologic resorbable scaffold. *Diabetes* 65(5):1350–61
132. Salvay DM, Rives CB, Zhang X, Chen F, Kaufman DB, et al. 2008. Extracellular matrix protein-coated scaffolds promote the reversal of diabetes after extrahepatic islet transplantation. *Transplantation* 85(10):1456–64
133. Hlavaty KA, Gibly RF, Zhang X, Rives CB, Graham JG, et al. 2014. Enhancing human islet transplantation by localized release of trophic factors from PLG scaffolds. *Am. J. Transplant.* 14(7):1523–32
134. Matsushima H, Kuroki T, Adachi T, Kitasato A, Ono S, et al. 2016. Human fibroblast sheet promotes human pancreatic islet survival and function in vitro. *Cell Transplant.* 25:1525–37
135. Medina JD, Alexander M, Hunckler MD, Fernández-Yagüe MA, Coronel MM, et al. 2020. Functionalization of alginate with extracellular matrix peptides enhances viability and function of encapsulated porcine islets. *Adv. Healthc. Mater.* 9(9):2000102
136. Llacua A, De Haan BJ, Smink SA, De Vos P. 2016. Extracellular matrix components supporting human islet function in alginate-based immunoprotective microcapsules for treatment of diabetes. *J. Biomed. Mater. Res. A* 104(7):1788–96
137. Paez-Mayorga J, Capuani S, Hernandez N, Farina M, Chua CYX, et al. 2020. Neovascularized implantable cell homing encapsulation platform with tunable local immunosuppressant delivery for allogeneic cell transplantation. *Biomaterials* 257:120232
138. Smith KE, Purvis WG, Davis MA, Min CG, Cooksey AM, et al. 2018. In vitro characterization of neonatal, juvenile, and adult porcine islet oxygen demand, β-cell function, and transcriptomes. *Xenotransplantation* 25(6):e12432
139. Emamaullee JA, Shapiro AMJ, Rajotte RV, Korbutt G, Elliott JF. 2006. Neonatal porcine islets exhibit natural resistance to hypoxia-induced apoptosis. *Transplantation* 82(7):945–52
140. Farina M, Ballerini A, Fraga DW, Nicolov E, Hogan M, et al. 2017. 3D printed vascularized device for subcutaneous transplantation of human islets. *Biotechnol. J.* 12(9):1700169
141. Komatsu H, Gonzalez N, Ortiz J, Rawson J, Omori K, et al. 2021. Early-phase luciferase signals of islet grafts predicts successful subcutaneous site transplantation in rats. *Mol. Imaging Biol.* 23(2):173–79
142. Shi W, Pawlick RL, Bruni A, Rafiei Y, Pepper AR, et al. 2016. Photoacoustic imaging of angiogenesis in a subcutaneous islet transplant site in a murine model. *J. Biomed. Opt.* 21(6):066003
143. Patel SN, Ishahak M, Chaimov D, Velraj A, LaShoto D, et al. 2021. Organoid microphysiological system preserves pancreatic islet function within 3D matrix. *Sci. Adv.* 7(7):eaba5515
144. Jiang K, Chaimov D, Patel SN, Liang J-P, Wiggins SC, et al. 2019. 3-D physiomimetic extracellular matrix hydrogels provide a supportive microenvironment for rodent and human islet culture. *Biomaterials* 198:37–48
145. Farina M, Chua CYX, Ballerini A, Thekkedath U, Alexander JF, et al. 2018. Transcutaneously refillable, 3D-printed biopolymeric encapsulation system for the transplantation of endocrine cells. *Biomaterials* 177:125–38