



Safe host integration in islet tissue engineering for Type 1 Diabetes Mellitus: emerging technologies and a constraint-guided hybrid islet framework

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Abstract

Type 1 diabetes mellitus (T1DM) results from autoimmune destruction of pancreatic β cells and requires lifelong insulin replacement. Islet transplantation can restore endogenous insulin secretion, but donor scarcity, immune rejection, early graft loss, inadequate oxygen delivery, and fibrosis limit its application. This review examines conventional and emerging islet tissue-engineering approaches and proposes a conceptual hybrid bio-artificial islet system (HBAIS). A heuristic constraint-exchange framework compares the functions gained from successive technological additions with their associated risks, overlapping benefits, and operational dependencies. The analysis identifies hypoimmune stem cell-derived β cells within a retrievable, transport-permissive interface, supplemented by temporary oxygen generation, as a provisional core configuration. Optogenetic amplification and synthetic protocell-mediated vascular support remain conditional additions when intrinsic insulin responsiveness or host vascular integration is inadequate. The review also proposes auxiliary donor-antigen-matched decoys, including lymph-node grafts, retrievable implants, and intraportal islets, as potential means of relocating immune interactions away from the principal graft. Their ability to intercept immune effectors or promote donor-specific regulation, rather than amplify sensitization, requires experimental evaluation. The framework and integrated system remain unvalidated, but provide a structured basis for investigating β -cell replacement architectures whose added complexity is justified by functional benefit.

Keywords

Type 1 Diabetes Mellitus, Islet Tissue engineering, Islet transplantation, Regenerative medicine, Stem cell-derived β cells, Hypoimmune cells, Oxygen-generating biomaterials, Optogenetics, Islet organoids, Synthetic biology, Protocell, Bioartificial pancreas

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1. Introduction

Type 1 diabetes mellitus (T1DM) is an autoimmune disease affecting approximately 9.1 million people worldwide. Diabetes overall accounts for ~ 12% of global health expenditure (1). T1DM was formerly known as “juvenile diabetes.” Although genetic and environmental factors contribute to T1DM, the precise mechanisms that trigger its onset remain unclear (2). The human leukocyte antigen (HLA) region on chromosome 6 has been recently studied and identified as a significant susceptibility locus relating to the disease (3). The standard treatment involves lifelong exogenous insulin replacement, delivered through subcutaneous

injections or an insulin pump, to regulate blood glucose levels. Although insulin therapy helps patients achieve glycemic control, it can impose ongoing discomfort, psychological stress, and financial burdens (4). Insulin therapy also carries a risk of hypoglycemia. Hybrid closed-loop systems adjust insulin delivery in response to glucose measurements, while long-acting basal insulin provides sustained background coverage; both approaches can help reduce this risk (5-7). Additional therapies are being investigated to preserve β -cell function and reduce immune-mediated destruction (3,8) (Table 1).

Table 1. Summary of the benefits and limitations of current drugs designed to enhance survival of the β -Cells.

Therapy Class	Primary Function & Benefits	Significant Limitations & Issues
Anti-CD3	Targets specific autoreactive T-cells to slow the destruction of β cells and delay disease onset.	Effects are limited and not permanent.
B-cell Targeting	Reduces autoantibody-producing B cells to protect islets.	Fails to provide sustained long-term disease control.
Broad T-cell Depletion	Suppresses the general T-cell population to halt the autoimmune attack.	Outcomes are inconsistent across patient populations.
Memory T-cell Targeting	Eliminates tissue-resident memory T-cells; shows trends toward β cell preservation.	Chronic autoimmunity remains difficult to fully eradicate.
Non-depleting Modulation	Prevents T-cell activation without removing the immune cells entirely.	Does not successfully halt the progression of the disease.
Cytokine-directed	Blocks inflammatory signaling molecules to prevent death of islets.	Human trials have shown inconsistent efficacy.
Antigen-specific Vaccines	Attempts to retrain the immune system to tolerate β cells without systemic suppression.	Currently lacks the ability to restore lost cell mass.
Amylin	Suppresses glucagon to assist in glucose regulation.	Limited by side effects such as nausea and increased hypoglycemia risk.
SGLT Inhibitors	Promotes urinary glucose excretion to lower blood sugar.	Effective but carries a high risk of diabetic ketoacidosis.
Verapamil	Reduces β cell stress and supports survival signals within the cell.	Clinical evidence is currently limited for broad application.
Metformin / GLP-1 RAs	Improves insulin resistance, weight loss, and reduces hepatic glucose production.	Provides only modest benefits, currently not considered disease-curing.

However, these therapies do not restore the β -cell mass lost in T1DM, a central limitation discussed later in this work (9). Consequently, islet transplantation has emerged as an alternative treatment by restoring β -cell mass through replacement of damaged islet cells. This method, however, faces limitations, such as donor shortages and immune rejection. Regenerative medicine offers approaches to address these limitations by generating stem cell-derived β cells, using growth factors to support cell survival and function, and applying tissue engineering strategies to improve graft survival. Stem cell-derived cells could reduce reliance on donor islets, while improved graft survival could lower the cell quantities required for transplantation. Together, these approaches aim to address donor scarcity and restore the β -cell mass lost in T1DM. This review outlines the pathophysiology of T1DM, examines conventional islet tissue-engineering strategies and their limitations, and evaluates emerging technologies for improving graft survival and function. It develops a heuristic constraint-transfer model that compares the severity-weighted biological constraints reduced by each intervention with the new risks and dependencies it introduces. Applied to progressively more complex graft configurations, the model identifies overlapping functions and the incremental contribution of each added component, helping distinguish a provisional core configuration from conditional enhancements. Finally, the review examines unresolved challenges to durable β -cell replacement.

2. Role of the islet in Type 1 Diabetes Mellitus (T1DM)

Insulin is a vital polypeptide hormone secreted by β cells in the islets of Langerhans of the pancreas. After a meal, dietary carbohydrates are digested into simple sugars, including glucose, which enters the bloodstream and stimulates insulin release. Insulin promotes glucose uptake, particularly in muscle and adipose tissue, and supports its use for energy or storage as glycogen in the liver and skeletal muscles (10). Along with glucagon, which promotes hepatic glucose production through glycogen breakdown and gluconeogenesis, insulin is necessary for maintaining blood glucose homeostasis.

In T1DM, autoimmune destruction of pancreatic β cells causes insulin deficiency and hyperglycemia, producing symptoms such as excessive thirst and fatigue. Severe insulin deficiency can lead to diabetic ketoacidosis and coma. Chronic hyperglycemia contributes to cardiovascular disease and damages small blood vessels and nerves, causing complications such as nephropathy, retinopathy, and neuropathy. Neuropathy may produce numbness in the limbs and impair digestive function (11). Because β -cell loss underlies insulin deficiency, a direct therapeutic approach aims to restore functional β -cell mass and glucose-responsive insulin secretion.

3. Current islet replacement therapies for the treatment of T1DM

3.1 Islet transplantation

Islet transplantation aims to restore functional β -cell mass by introducing insulin-producing cells. Four approaches are discussed below: allogeneic islets from human donors,

xenogeneic islets from other species, autologous islets from the patient, and stem cell-derived islets. Their suitability differs; autologous transplantation, in particular, is generally unsuitable for established T1DM because few functional β cells remain.

3.1.1 *Allogeneic islets*

Allogeneic islets are clusters of pancreatic endocrine cells obtained from human donors and transplanted into recipients. Following infusion into the portal circulation, an instant blood-mediated inflammatory reaction can cause substantial early graft loss, while subsequent immune rejection further threatens graft survival. The Edmonton Protocol, introduced in 1999, improved transplantation outcomes through a standardized approach incorporating steroid-free immunosuppression. However, high doses of drugs to suppress the immune system (immunosuppressants) are necessary to prevent the body from rejecting the donor islets (12). Despite these advances, transplantation remains limited, where even when patients and donors share similar HLA types (proteins used by the immune system to recognize cells), insulin independence is not consistently achieved. Additionally, a lack of available donors limits the number of possible treatments (13).

3.1.2 *Xenogeneic islets*

Islet xenotransplantation uses insulin-producing cells from another species, commonly pigs, whose insulin differs from human insulin by one amino acid. Donor pigs can be selectively bred, maintained under pathogen-controlled conditions, and genetically modified to reduce immune rejection and infectious risks, including

those associated with porcine endogenous retroviruses. A 2007 study reported an approximately 30% reduction in a patient's insulin requirement over 12 weeks following transplantation of encapsulated porcine islets, suggesting potential therapeutic benefit. However, immune rejection, infectious risks, long-term graft safety, and ethical concerns surrounding animal use and genetic modification remain barriers to widespread adoption (13).

3.1.3 *Autologous islets*

Autologous islet transplantation involves isolating islets from a patient's surgically removed pancreas and reinfusing them, typically into the liver through the portal vein. Because the cells originate from the patient, immunosuppression to prevent allogeneic rejection is unnecessary (8). This procedure is primarily used alongside pancreatic removal for conditions such as severe chronic pancreatitis. It is generally unsuitable for established T1DM because autoimmune destruction leaves insufficient functional β -cell mass for transplantation.

3.2 Stem cell-derived islet cells

Stem cell-derived β cells offer a promising approach to restoring insulin production. Human embryonic stem cells (hESCs) and induced pluripotent stem cells (iPSCs), generated by reprogramming adult cells, can differentiate into multiple cell types. Through controlled exposure to signaling molecules that regulate developmental pathways and transcription factors, these cells can be guided toward an insulin-producing β -cell identity.

However, iPSC-derived β cells may not fully reproduce the characteristics of mature native β cells because differentiation and functional maturation can remain incomplete. In a murine study, undifferentiated and partially differentiated iPSCs formed teratomas at all injection sites, whereas fully differentiated cells did not form tumors under the conditions examined. These findings underscore the importance of eliminating residual undifferentiated cells before transplantation (14). Reported post-transplantation cell survival ranges from 5% to 47%, reflecting substantial graft loss (8). Contributing factors include inadequate vascularization and oxygen supply, mechanical injury during transplantation,

immune-mediated damage, and incomplete cellular maturation. Together, these barriers limit the survival and function of stem cell-derived islet grafts in T1DM.

4. Engineering strategies to protect transplanted islet cells

Transplanted islets face several barriers to survival and function, including immune-mediated injury, fibrosis around the graft, and inadequate nutrient and oxygen supply. Stem cell-derived grafts may also exhibit incomplete functional maturation (8). The following sections examine tissue-engineering strategies designed to mitigate these challenges and improve graft survival and function.

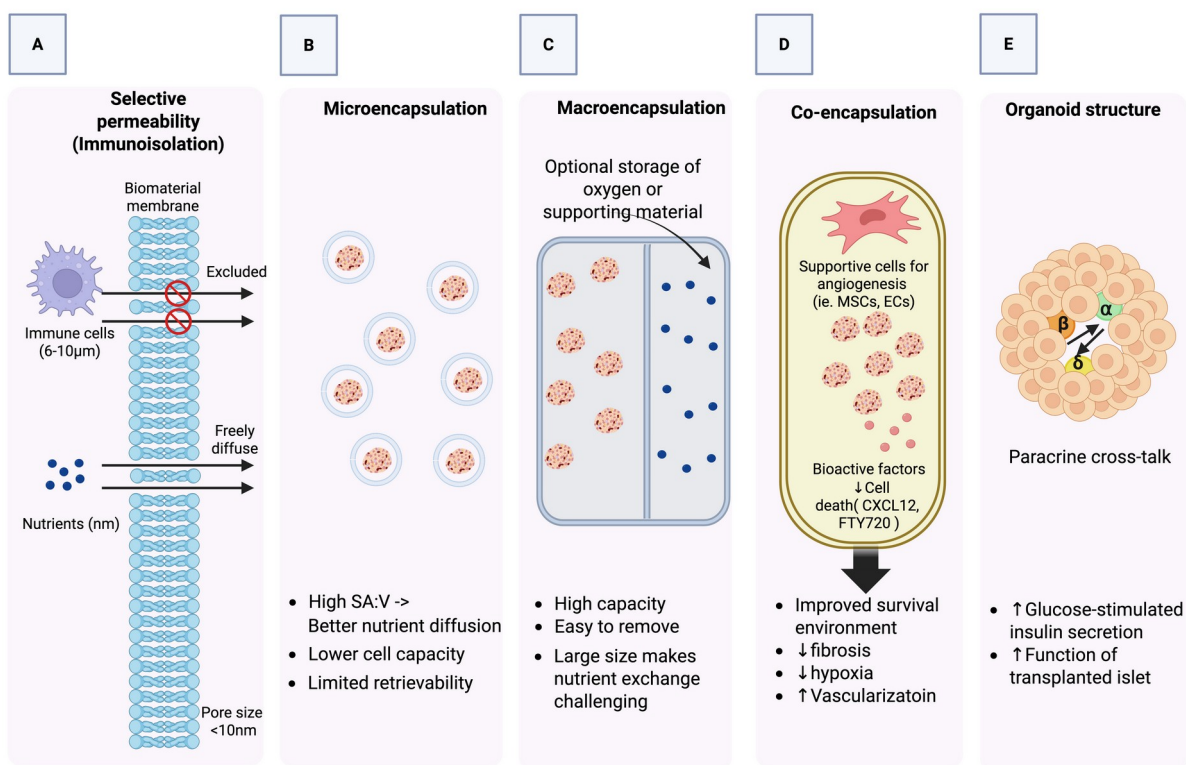


Figure 1. Encapsulation and islet tissue-engineering strategies to improve survival and function of islet post-transplantation. Figure created using [Biorender.com](https://biorender.com)

4.1 Encapsulation

A major limitation of allogeneic and xenogeneic islet transplantation is the need for immunosuppression, which carries long-term risks and can affect patients' quality of life. Encapsulation within immunoprotective biomaterials has therefore been extensively investigated as a strategy to reduce immune exposure while minimizing material-induced inflammation. These biomaterials are engineered with pores small enough to prevent immune cell infiltration while permitting the diffusion of essential nutrients through the encapsulating membrane in order to maintain graft's survival. Immune cells typically range from 6 μm to 10 μm in diameter, whereas organic metabolites range from 0.05 nm to 1 nm and proteins from 2 nm to 10 nm (15). Excluding micrometer-sized immune cells does not require pores below 10 nm. More restrictive barriers may additionally limit passage of soluble immune factors, but must preserve adequate transport of oxygen, nutrients, glucose, and insulin. Encapsulation strategies are generally classified by size. Microencapsulation encloses individual islets or small groups of islets within small capsules, whereas macroencapsulation uses larger devices, typically millimeters to centimeters in size, to accommodate greater numbers of islets (Figure 1A).

4.1.1 Microencapsulation

Microencapsulation encloses one or a few islets within a spherical biomaterial capsule, typically 300–1000 μm in diameter. The capsule limits direct contact with immune cells, while its relatively high surface-area-to-volume ratio facilitates diffusion of oxygen, nutrients,

glucose, and insulin between the graft and surrounding tissue. A controlled study showed that alginate microencapsulated human islets maintained normoglycemia for 134 ± 17 days in immunocompetent diabetic mice, whereas unencapsulated islets were rejected within 2–7 days (16). Microcapsule size and uniformity can be controlled using droplet microfluidics and other fabrication techniques. In coaxial airflow systems, gas flows alongside the liquid at the nozzle, breaking it into droplets through aerodynamic forces and surface tension. Other approaches include microfluidic chips, centrifugal droplet generation, electrostatic spraying, and conformal coating, which deposits a thin biomaterial layer directly around the islets rather than enclosing them within larger capsules (17) (Figure 1B).

4.1.2 Macroencapsulation

Macroencapsulation refers to capsule devices with diameters ranging from millimeters to centimeters. The disadvantages of a larger size include increased thickness of the biomaterial membrane and a reduced surface-area-to-volume ratio, which makes diffusion and nutrient exchange more difficult (16). On the other hand, macroencapsulation offers the advantages of a large cell capacity, enabling islets to be grown more effectively, as well as enhanced retrievability. With the risks of tumour formation, the ability to surgically remove the entire graft, should complications arise, is a critical safety feature. Additionally, the capsule's large size allows for the installation of additional devices, thereby enhancing the survivability of the islet grafts. Devices such as the β -Air, an implantable bio-artificial pancreas

by Beta-o2 Technologies, can be implanted to deliver oxygen (18) (Figure 1C).

4.1.3 Biomaterials

Biomaterials play a key role in these engineering strategies, providing a microenvironment that can shield transplanted islets from immune attack while allowing nutrient and oxygen diffusion. Currently, alginate hydrogel remains the earliest and most reliable choice of biomaterial for protecting islets against the immune attack. This is due to its high biocompatibility and non-degradable properties (17). Derived from brown seaweed, the gel is formulated via ionic crosslinking with Ca^{2+} and Ba^{2+} , enabling reversible ionic properties. The structure of alginate contains β -d-mannuronic acid (M) and α -l-guluronic acid (G), where increasing the G blocks can increase stiffness, create a denser structure, and effectively shrink the pore sizes. This allows the alginate hydrogel's physical structure to be manipulated with ease, allowing for adjustable pore sizes (19).

In addition to alginate hydrogel, other natural and synthetic materials are being explored, including chitosan, silk protein, agarose, PEG, and polyacrylate (13). Dual layers of encapsulation are also employed in Polyethylene glycol (PEG), for instance, where the inner layer can be coated with laminin peptides to promote insulin secretion and the outer layer for protection (17). Porous and hydrophobic polymer materials in which air is trapped can also be used to create an "oxygen reservoir". Therefore, this allows for increased oxygen diffusion and transport into a region (20).

4.2 Co-encapsulation

Co-encapsulation incorporates supportive cells or bioactive substances alongside islets within an encapsulating material to improve graft survival and function. Kerby et al. co-encapsulated mouse islets with mesenchymal stem cells (MSCs) in alginate microcapsules and transplanted them into diabetic mice. At six weeks, diabetes reversal was reported in 71% of recipients receiving co-encapsulated grafts, compared with 16% receiving encapsulated islets alone. Co-encapsulation also increased glucose-stimulated insulin secretion and insulin content, demonstrating that supportive cells can improve graft function even in a nonvascularized encapsulation model (21).

Maintaining adequate oxygen and nutrient delivery remains a major challenge, particularly before sufficient vascular support develops around the graft. MSCs secrete proangiogenic and immunomodulatory factors that may support vascular development and islet survival (22). Other supportive components, including endothelial cells, microvascular fragments, and amniotic epithelial cells, have been investigated for their ability to promote vascular organization, cell survival, and favorable interactions with surrounding tissue. Bioactive substances can provide additional protection: bilirubin offers antioxidant and anti-inflammatory effects, while CXCL12 and FTY720 can modulate immune-cell recruitment or trafficking to reduce graft-directed inflammation (15) (Figure 1D). These benefits depend on the encapsulating material and graft configuration, which must permit adequate molecular transport.

4.3 Islet Organoids

Beyond encapsulation, islet organoids offer another tissue-engineering approach. These three-dimensional structures can be generated from stem cell-derived pancreatic cells that self-aggregate and organize under controlled culture conditions (23). Organoids are formed before transplantation rather than assembling in the bloodstream. Decellularized extracellular matrix (dECM), combined with biochemical cues, can provide a supportive environment for cellular organization, growth, and maturation.

A key advantage of islet organoids is their ability to support cell-to-cell interactions within a three-dimensional endocrine microenvironment. Paracrine signaling among β cells and other endocrine cells, particularly glucagon-secreting α cells and somatostatin-secreting δ cells, helps coordinate glucose-responsive insulin secretion. Recreating these interactions can support the maturation and function of stem cell-derived β -like cells. Organoid organization therefore aims to reproduce local islet architecture and signaling, rather than relying on communication between separate transplanted islets (22).

Despite their organized structure, transplanted islet organoids remain vulnerable to immune-mediated injury. Incorporating supportive cells or immunomodulatory factors may help reduce this injury and improve graft survival (22,24). Combining organoids with encapsulation offers a complementary strategy: the organoid preserves local cellular organization and signaling, while the surrounding biomaterial limits immune exposure. This protection must be balanced against adequate transport of

oxygen, nutrients, glucose, and insulin (Figure 1E).

5. Irreducible design constraints in long-term β -cell replacement

Despite advancements in islet tissue engineering, several constraints hinder the usage of islet transplantation as a true cure for T1DM. Long-term β -cell replacement therefore requires the simultaneous consideration of the major constraints.

5.1 Immune compatibility

A major limitation of allogeneic and stem cell-derived β -cell transplantation is immune-mediated graft injury. Immunosuppressive therapy can reduce rejection but carries long-term risks. Encapsulation limits direct contact between host immune cells and the graft, although restrictive barriers can also impede molecular transport. This tradeoff suggests that reducing the immunogenicity of transplanted cells could improve immune compatibility while allowing a more transport-permissive protective interface.

5.2 Mass transport and oxygenation

Transplanted islet grafts require a continuous supply of oxygen, glucose, and nutrients, while secreted insulin must diffuse outward. Immediately after transplantation, vascular support is often inadequate, leaving the graft largely dependent on passive diffusion as host neovascularization develops (20,25). Oxygen delivery is therefore constrained by diffusion distance and cellular oxygen consumption (25). A critical oxygen partial pressure (pO_2) of 2.43 ± 0.08 mmHg has been reported under the conditions examined; lower oxygen availability

can compromise cell survival, particularly at the graft center, and cause central necrosis (20). Graft injury can contribute to inflammation, while the foreign-body response to encapsulating materials can produce pericapsular fibrotic overgrowth (PFO). This collagen-rich barrier further restricts oxygen and nutrient transport and delays glucose sensing and insulin delivery (15).

5.3 Long-term safety and stability

Stem cell-derived grafts introduce safety concerns distinct from those associated with mature donor islets. Residual undifferentiated or partially differentiated pluripotent cells may retain tumorigenic potential, making rigorous control of differentiation and long-term monitoring essential (14). Surrounding biomaterials introduce additional risks, including foreign-body responses, fibrosis, degradation, toxic reaction byproducts, and mechanical failure. Graft safety therefore requires sustained assessment of interactions among transplanted cells, engineered materials, and the host, together with reliable containment and retrievability throughout implantation.

5.4 Donor scarcity and early graft loss

The limited supply of donor pancreatic tissue restricts access to islet transplantation for the large population affected by T1DM (1). Early graft loss compounds this shortage: the instant blood-mediated inflammatory reaction (IBMIR) can destroy up to 50% of transplanted islet mass (26). Reported post-transplantation cell survival rates range from 5% to 47% (8), increasing the cell quantities required to restore adequate insulin secretion. Some recipients therefore require islets from multiple donors or repeated

infusions to achieve insulin independence, further straining the available tissue supply.

6. Emerging technologies

Current islet tissue-engineering approaches face limitations that compromise graft survival and treatment success. The next section examines emerging strategies that may address these limitations and improve graft survival and function.

6.1 Oxygen generation

Strategies to mitigate hypoxia include co-encapsulation with angiogenic factors, but vascularization takes time to develop. Supplemental oxygen can provide support during this interval. The β -Air device, introduced in the macroencapsulation section, uses refillable oxygen chambers to reduce reliance on host oxygen delivery (25). Complementary approaches generate oxygen locally using oxygen-releasing materials, as discussed below.

Peroxide and percarbonate compounds have been recognized for their ability to produce a stream of oxygen when exposed to water. For example, calcium peroxide can produce oxygen when exposed to water, which is plentiful in the human body. In an experiment conducted by Pedraza et al., calcium peroxide was encapsulated within the biomaterial PDMS. PDMS enabled the controlled release of oxygen from calcium peroxide, thereby maintaining continuous diffusion and reactivity control. Under the reported experimental conditions, the system provided sustained oxygen generation over several weeks, demonstrating the potential

to provide oxygen support during the early post-transplantation period (25,27).

Electrolysis can generate oxygen from water for delivery to encapsulated islet grafts. This approach requires reliable separation of hydrogen and oxygen, controlled oxygen delivery, and management of electrical and chemical risks to support graft function safely (20). Photosynthetic microorganisms offer another potential oxygen source. Co-encapsulation with a suitable light source could enable local oxygen production, although its effectiveness depends on adequate illumination, microbial viability, and compatibility with the graft and host.

An alternative to the refillable oxygen reservoir used in β -Air is a controlled conduit connecting the graft region to atmospheric air, which contains approximately 21% oxygen. Such a system could potentially supply oxygen by diffusion without repeated reservoir refilling. However, adequate delivery would depend on the oxygen gradient and transport resistance, while any connection across the skin would require protection against infection and reliable long-term maintenance.

Genetic modification of islet cells has also been investigated as a means of improving tolerance to hypoxia. Cytochrome b5, encoded by *CYGB*, is implicated in intracellular oxygen handling and protection against oxidative stress. In a rat transplantation model, grafts overexpressing *CYGB* maintained normoglycemia for up to 60 days, compared with 14 days in controls (20).

6.2 Hypoimmune cell engineering

Recent advances in hypoimmune cell engineering aim to reduce immune recognition of transplanted grafts through genetic modification, potentially reducing or eliminating the need for systemic immunosuppression. The following section outlines the mechanisms of immune recognition underlying these approaches.

6.2.1 Main types of immune antigens

Immune recognition often occurs via antigens on the surface of molecules, namely, major histocompatibility complex molecules. In humans, these are known as the human leukocyte antigen (HLA) proteins. The HLA proteins can be separated into two classes (26).

[1] HLA class I: Regulated by β 2 microglobulin (B2M) and TAP1/2, it is expressed on all nucleated cells and presents the “information” for the adaptive immune system’s CD8+ cytotoxic T-cells (killer T-Cells), whether the cell is cancerous or pathogenic. To avoid being attacked, some tumors and virus-infected cells downregulate these glycoproteins. Hence, the innate immune system’s natural killer (NK) cells scan for cells that exhibit abnormally low HLA class I.

[2] HLA class II: Controlled by RFXANK, RFX5, RFXAP, and the class II transactivator (CIITA), it is principally expressed on professional antigen-presenting cells that are specialized cells to bridge the innate and adaptive immune system, such as B cells, but are also expressed upon activation of inflammatory cytokines. For instance, immune cells secrete interferon gamma (IFN- γ), which in turn induces the expression of these glycoproteins.

These glycoproteins bind antigens from foreign cells and are recognized by CD4⁺ T helper cells, which activate other immune cells. To avoid autoimmunity, CD4⁺ regulatory T cells (Tregs) additionally regulate CD4⁺ T helper cells (28).

Some essential cells, such as those in the eyes, are granted privileged status and are spared immune recognition. Inspired by this, scientists can create hypoimmune cells by deleting or silencing HLA genes, evading immune attacks (26).

6.2.2 Bioengineered hypoimmune cells

Hypoimmune engineering modifies pathways involved in graft recognition and immune-

mediated injury. B2M disruption reduces surface expression of classical HLA class I molecules, while CIITA disruption reduces HLA class II expression. Because loss of HLA class I can provoke NK-cell-mediated killing, complementary strategies include inhibitory ligands such as HLA-E, HLA-G, and CD47. Other approaches employ PD-L1 or CTLA4-Ig to inhibit T-cell responses, or delete NK-cell-stimulating ligands such as B7-H3 and CD155. These interventions reduce selected immune pathways, but their effectiveness depends on the engineered cell type and host immune environment (28).

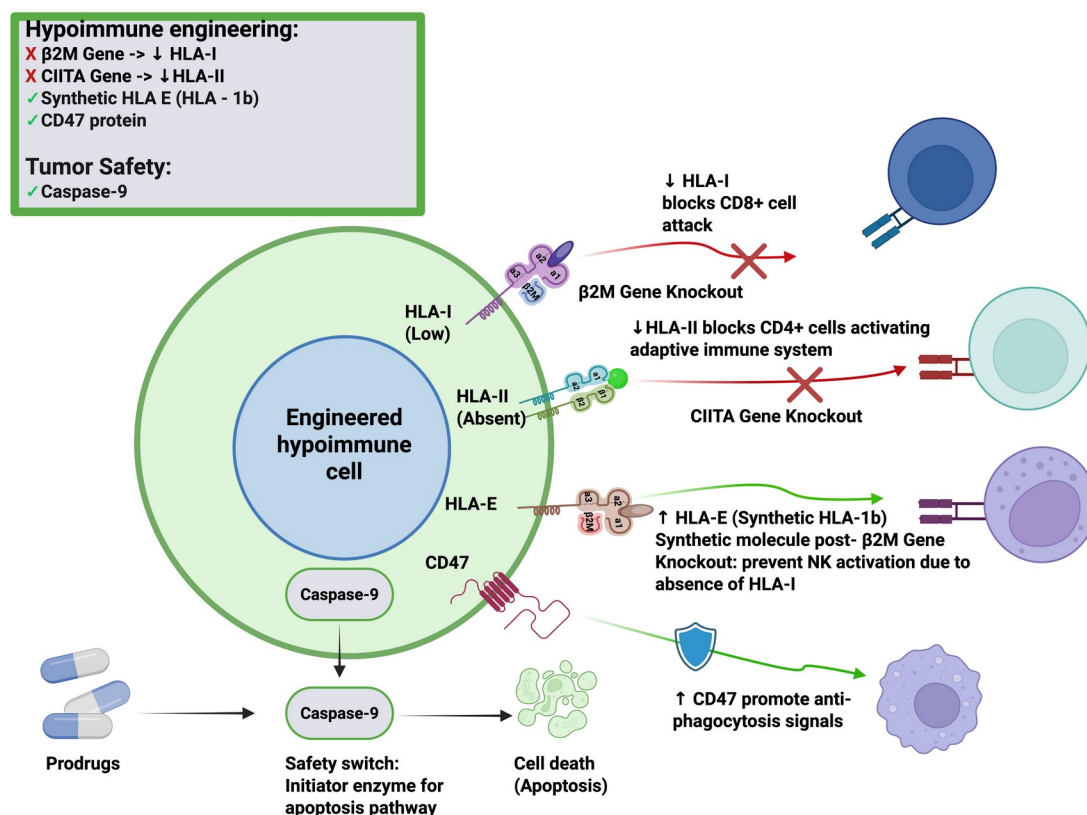


Figure 2. Molecular modifications used to create islets that can evade immune detection. Figure created using [Biorender.com](https://www.biorender.com)

Complete depletion of HLA class II molecules can reduce direct CD4⁺ T cell-mediated immune responses. This can be achieved by manipulating the CIITA gene; simultaneous targeting of B2M and CIITA has been shown to effectively eliminate both HLA class I and class II. Alternatives, such as directly targeting the HLA class II gene, have also shown potential, as HLA class II gene expression is regulated by the CIITA pathway. Complete removal of HLA class I can trigger natural killer (NK) cell responses through “missing-self” recognition. Hypoimmune strategies may therefore incorporate additional inhibitory signals. Non-classical HLA class I molecules, such as HLA-G, can inhibit immune-cell activity and support regulatory T-cell responses. CD47 overexpression provides a complementary “don’t eat me” signal that may reduce innate immune-mediated destruction of HLA-deficient cells, although it does not confer complete immune protection (Figure 2).

6.3 Gene editing technologies

A major safety concern is tumor formation from residual undifferentiated human pluripotent stem cells (hPSCs). Inducible suicide-gene systems offer a potential means of eliminating engineered cells if abnormal growth develops. Some systems convert an administered prodrug into a cytotoxic compound, whereas others activate programmed cell death directly. For example, inducible caspase-9 (iCasp9) is a drug-activated safety switch that triggers apoptosis directly rather than converting a prodrug into a cytotoxic compound (29). Such systems provide an additional safeguard but should not be

assumed to eliminate every potentially harmful cell.

6.4 Optogenetics and precision control

Islet grafts rely on intrinsic glucose sensing, which may be impaired by cellular immaturity or hypoxia at the transplantation site. Optogenetic approaches offer externally controlled stimulation of insulin secretion. In systems using light-sensitive ion channels, illumination alters membrane potential and promotes calcium influx, triggering the release of stored insulin (30–31). These approaches require viable insulin-producing β cells and therefore complement β -cell replacement rather than substitute for it. Their effectiveness also depends on adequate insulin stores, metabolic support, and control that prevents inappropriate secretion at low glucose levels.

Newer optogenetic systems aim to control gene expression directly via light-activated transcription factors, enabling programmable production of therapeutic proteins rather than simply triggering their release. A widely used example is the LightOn system (Figure 3). By incorporating fungal genes and proteins, specific light wavelengths trigger dimerization at specific regions of the structure, promoting and recruiting RNA Polymerase, ultimately inducing transcription of the insulin transgene. Other wavelengths that are more tissue-penetrable, such as red and green light, have also been developed, including the FRL-controlled system and Glow Control (30). Additionally, self-sustaining systems that do not require external power for the light source are being developed. The BphS FRL system was found to

restore normoglycemia and convert energy from respiration into electricity to power the LED system. Recent advances have enabled external, light-free systems, such as the use of NanoLuc, which generates light internally within the LuminON system (32). The LuminON system

demonstrated maximal transgene expression with sustained expression lasting 2–3 hours. Nanomaterials that turn NIR light into higher energy light have also been explored to be used in optogenetic systems.

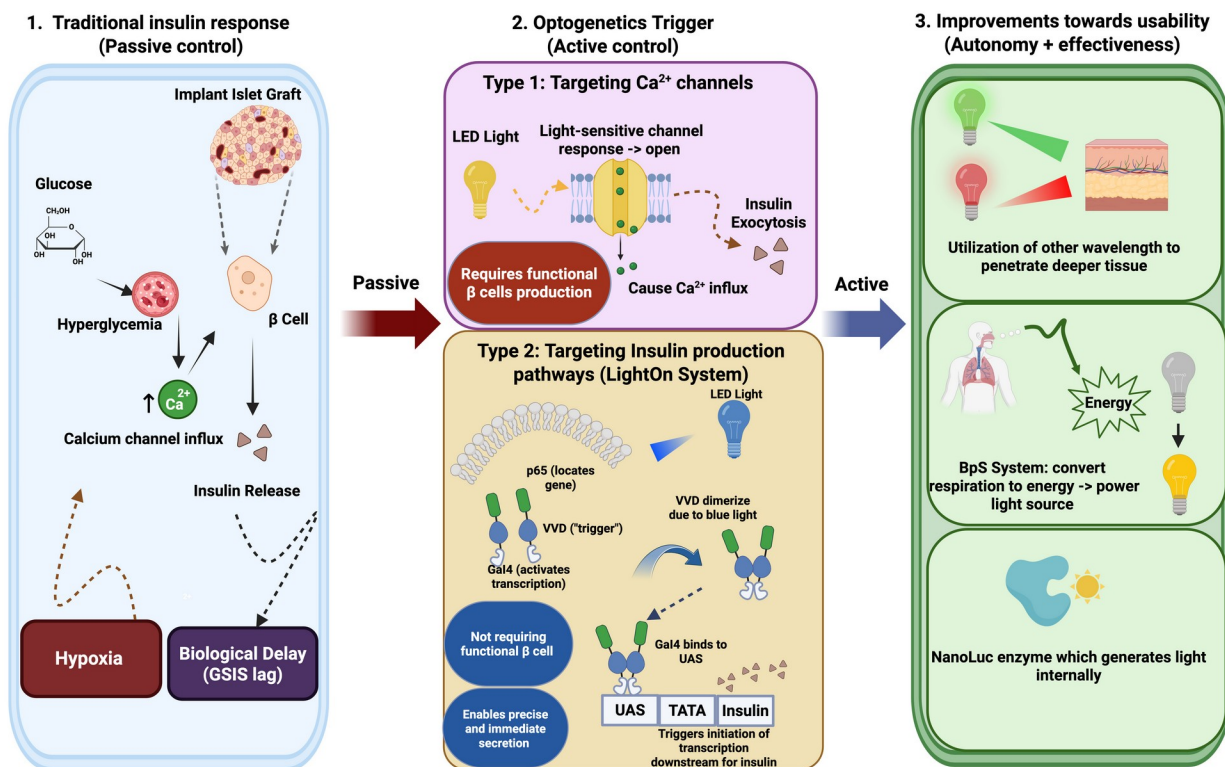


Figure 3. Contrasting traditional glucose sensing and active, light-triggered insulin production systems.

Figure created using [Biorender.com](https://biorender.com)

Optogenetic approaches face limitations that vary with their mechanism, including delays in transcription-based responses, variable activation duration, unintended stimulation by ambient light, and potential immunogenicity of introduced proteins such as VVD. Alternatives include light-responsive compounds, while other approaches amplify endogenous GSIS pathways rather than initiate insulin synthesis,

although they may still require genetically introduced actuators. Carefully designed light-delivery systems could improve control of insulin secretion and reduce reliance on injections, but do not inherently eliminate the need for glucose monitoring (30).

6.5 “Cell-Free” revolution and synthetic protocells

A departure from conventional tissue engineering is the use of synthetic cell-like compartments to reproduce selected cellular functions. These structures, broadly termed protocells, contain reactants within membrane-bound or membraneless compartments, enabling localized biochemical reactions. They need not rely on oxidative phosphorylation, although their activity depends on substrate availability, compartment stability, and preservation of the incorporated reaction network. They may therefore perform selected functions under conditions that compromise living cells, without reproducing their full metabolic capabilities (33,34).

Protocell design balances structural stability with permeability. Giant unilamellar vesicles (GUVs) provide simplified lipid-bilayer compartments, while hybrid lipid-polymer vesicles offer opportunities to tune membrane properties and functional versatility (35). Other platforms include polymersomes for structural durability, proteinosomes for compartmentalized enzyme cascades, and caposomes for loading and delivering multiple agents. Membraneless coacervates provide an alternative means of concentrating reactants and supporting reactions that would be inefficient in dilute solution (36).

The proof of concept for artificial cell functionality is demonstrated by the ability to perform cell-free protein synthesis (CFPS), where proteins are able to be produced outside intact living cells, using supplied DNA or RNA templates under a cell-free environment (37). This technology functions by combining cell lysate, genetic templates, as well as supporting

substances, such as amino acids and excess nucleotides, achieving evidential success and able to produce a maximum protein yield of ~9.7 mg/mL. Although still under development, reports indicate promising results, including continuous production of 4 mg/mL of proteins for over 20 hours (38). Despite promising laboratory results, clinical translation requires scalable and reliable insulin production. Milligram-scale yields from current cell-free protein synthesis (CFPS) systems do not alone establish clinical adequacy; production must also meet requirements for sustained delivery, biological activity, purity, stability, and glucose-responsive dose control.

7. Proposed hybrid islet system

7.1 Integration of technologies and conceptual basis of HBAIS

Current islet tissue-engineering approaches face limitations that compromise graft survival and therapeutic efficacy. Restoring functional β -cell mass is a central goal of β -cell replacement therapies because its loss underlies insulin deficiency in T1DM (9). However, transplantation alone is insufficient to ensure durable function. After implantation, grafts face interconnected challenges, including immune-mediated injury, inadequate oxygen and nutrient delivery before vascularization develops, collagen-rich fibrosis arising from foreign-body responses, incomplete functional maturation, and safety risks associated with stem cell-derived tissues (8,15,20,26).

To address these concurrent barriers, this review proposes a conceptual hybrid bio-artificial islet system (HBAIS) that integrates selected

emerging technologies as complementary subsystems intended to support graft survival and function.

The limitations described above are not necessarily independent engineering problems requiring separate interventions. Immune protection, vascularization, and oxygen delivery are interconnected through the graft's interaction with the host environment. Traditional macroencapsulation restricts direct contact between transplanted cells and host immune cells. However, this physical barrier also limits vascular access and increases resistance to oxygen and nutrient transport, potentially causing hypoxia and cell death (15,20,39,40). Physical immunoisolation can therefore contribute to the transport and vascularization constraints that additional engineering interventions seek to resolve.

Hypoimmune engineering may reduce recognition through major host immune pathways, transferring part of the immunoprotective function from the physical barrier to the transplanted cells. If this protection is sufficient to permit closer host interaction, the need for restrictive physical immunoisolation may decrease. A more transport-permissive interface could then support closer vascular proximity and improve oxygen and nutrient exchange, while increasing dependence on the durability of cellular immune evasion.

The central principle of HBAIS is therefore to engineer the graft so that closer host integration becomes sufficiently safe, reducing the need to address immune protection, oxygen delivery, and vascular support as separate problems.

Adding subsystems increases biological and engineering complexity. The framework therefore compares configurations with and without each subsystem to assess whether addressing an upstream constraint reduces downstream requirements. Additional subsystems are retained only when they address a biologically important unresolved limitation and their benefit justifies the added burden.

7.1.1 Evidence-mapped constraint-transfer framework

The proposed HBAIS architectures have not been experimentally compared; therefore, this analysis cannot directly establish their relative performance from empirical measurements.

A simple heuristic scoring procedure makes the architectural comparison systematic, transparent, and reproducible, while retaining qualitative judgments about benefits and burdens. First, a fixed set of constraint domains is established to represent broad challenges to graft survival, function, and safety. Constraints describing the same underlying problem are grouped within a single domain to avoid double-counting. For example, “early hypoxia” and “inadequate early oxygenation” are treated as one constraint domain.

Second, each technology i is characterized by two sets drawn from the fixed constraint domains: R_i , comprising domains it reduces, and I_i , comprising domains it introduces or worsens. Each domain receives a severity weight: Minor = 1, Major = 2, and Critical = 3. Minor constraints primarily increase operational or manufacturing burden; major constraints may substantially impair efficacy or long-term

reliability; and critical constraints may cause graft failure or serious harm to the host. These heuristic weights are assigned to domains rather than individual technologies, ensuring consistent treatment of the same underlying constraint throughout the analysis. The domains and their assigned weights are presented in Table 2.

Table 2. Summary of the predefined constraint domains, their operational definitions, and assigned severity scores used in the SWCE framework.

Constraint domain	Definition	Severity Score
Immune compatibility	Cellular or humoral immune injury that threatens graft persistence.	Critical — 3
Early oxygenation	Oxygen availability during implantation and the pre-/early-vascularization period.	Critical — 3
Long-term mass transport	Sustained transport of oxygen, glucose, nutrients, and insulin after the early engraftment period.	Major — 2
Vascular integration	Establishment and maintenance of adequate host vascular proximity or perfusion.	Major — 2
Physiological insulin responsiveness	Appropriate glucose-responsive insulin secretion and secretory amplification.	Major — 2
Containment and retrievability	Localization of engineered cells and the ability to remove the graft when required.	Major — 2
Tumor/genomic safety	Tumor escape, genomic instability, or reduced immune surveillance of abnormal engineered cells.	Critical — 3
Material/chemical toxicity	Toxic reaction products, pH disturbance, excessive NO/peroxide exposure, or other material-associated chemical injury.	Major — 2
Fibrosis	Foreign-body-associated fibrotic deposition that impairs transport or host integration.	Critical — 3
External intervention dependency	Requirement for external light, substrates, replenishment, drugs, or other continuing input.	Minor — 1
Mechanical reliability	Structural degradation, deformation, or mechanical failure that impairs device integrity or performance.	Major — 2
Manufacturing/ regulatory complexity	Additional fabrication, genetic engineering, quality-control, or regulatory burden created by the intervention.	Minor — 1

From these values, the effect of an intervention is expressed using the Severity-Weighted Constraint Exchange (SWCE), where (X) represents the intervention or architecture being evaluated, (R_i) and (I_i) represent the sets of fixed constraint domains reduced or introduced by component (i), and ($W(c)$) is the fixed severity weight assigned to constraint domain (c). The union is hence applied to the domains before severity weights are added. This ensures that the same domain is counted only once when affected by multiple components, while different domains carrying the same severity weight remain separately counted.

$$SWCE(X) = \sum_{c \in \bigcup_{i \in X} R_i} W(c) - \sum_{c \in \bigcup_{i \in X} I_i} W(c)$$

A positive SWCE value indicates that the assigned severity weights of the constraint domains reduced exceed those introduced or worsened. A value of zero indicates an equal weighted balance, while a negative value indicates that introduced or worsened domains carry greater total weight than those reduced. These values represent heuristic balances rather than measured changes in biological risk.

In addition, the evidence supporting each technology–constraint relationship is classified to distinguish experimentally demonstrated effects from theoretically plausible relationships. Technology-level SWCE values characterize constraint exchanges and inform the architecture-level analysis. Evidence classifications do not alter SWCE; they indicate confidence in the assigned reductions or introduced burdens. Future research could mathematically operationalize these relationships using systematically extracted effect sizes, study quality, consistency, and relevance to the proposed graft architecture, rather than fixed domain weights alone.

After individual technologies are evaluated, the same constraint accounting is applied to each HBAIS architecture. For a single technology, (X) contains only that intervention; for a complete architecture, (X) contains all included components. The unions of reduced and introduced or worsened domains are calculated separately before their severity weights are summed. Thus, each domain receives at most one reduction credit and one burden penalty per architecture, even when multiple components affect it.

As each architecture is evaluated, the analysis then examines whether adding each subsystem improves the preceding architecture. The marginal constraint exchange value is hence adopted, where the value of technology I, comparing architecture B with the same architecture without component I, architecture A, $\Delta SWCE_I = SWCE_B - SWCE_A$. A positive value indicates that addition of the component produces additional net constraint reduction, a value of zero indicates no additional net severity-weighted constraint reduction, and a negative value indicates that its addition increases the overall constraint burden. This comparison is important because a technology may appear beneficial when considered independently but provide little additional value if an earlier component has already reduced the same constraint.

Finally, architecture selection follows a fixed sequence rather than simply choosing the largest SWCE score. Architectures unresolved critical constraints or an unresolved credible risk of catastrophic loss of graft containment are identified first. Among architectures in which critical risks have a significant mitigation, their architecture-level SWCE are then compared, where further on marginal $\Delta SWCE$ identifies whether the newest subsystem changes the number of constraints. Evidence strength, temporal role, and reversibility are lastly analyzed serving as qualitative checks on whether the scored relationship is sufficiently supported. A positive marginal SWCE identifies an additional constraint reduction but by itself is unable to establish that the subsystem is required in the minimum graft configuration. If two architectures remain equivalent after these

comparisons, the less complex architecture is retained.

7.2. Candidate components of HBAIS

7.2.1 Stem cell-derived β cells

The fundamental therapeutic component of HBAIS is a stem cell-derived β -cell graft. By providing insulin-producing cells to replace those lost through autoimmune destruction in T1DM, stem cell-derived grafts could reduce reliance on scarce donor islets (9).

However, stem cell-derived β cells may remain incompletely differentiated or functionally immature. Residual undifferentiated or partially differentiated iPSCs can also retain tumorigenic potential, including the capacity to form teratomas (14). These grafts remain vulnerable to host immune-mediated injury. Thus, restoring functional β -cell mass alone does not address immune rejection or the requirements for physical containment, retrievability, and safe host integration.

Hence, HBAIS utilizes hypimmune engineering to effectively reduce immune-mediated rejection, boosting survival rates of the cell graft. This includes knocking out B2M and CIITA to disrupt HLA Class I and II assembly and evade T-cells, while overexpressing CD47 to provide a dominant signal preventing macrophage phagocytosis and NK-cell mediated cytotoxicity, the graft may substantially reduce recognition through several major cellular immune pathways. As such, hypimmune engineering may transfer a substantial portion of immunoprotection from the surrounding physical material to the cellular

graft itself (26,28). However, the graft should not be considered completely invisible to the host immune system. Antibodies, cytokines, complement proteins, inflammatory mediators, incomplete immune evasion, and reduced immune surveillance of abnormal cells remain potential concerns (26,28,40,41). Hypimmune engineering therefore reduces selected immune constraints rather than eliminating the requirement for immune compatibility or long-term safety assessment.

7.2.2 Hypimmune engineering & physical interface

With the implementation of immunoprotective technologies, the edited cells can reduce recognition by major host immune pathways, leading to greater flexibility in selecting a thinner, more transport-permissive, and mechanically durable material. Importantly, the interface choice should not be neglected, as hypimmune technologies by themselves do not provide cell containment, control of graft geometry, mechanical protection, retrievability, or control of diffusion distance.

To maintain precise and reliable dynamics over time, the ideal material must possess long-term chemical stability, mechanical durability, and the ability to maintain a rigid yet highly tunable porosity without degrading. For example, spiral polyethersulfone (PES) membrane has been recently noted with its superior mechanical resilience and chemical stability compared to the prone-to-degradation nature of alginate. Using a 0.5 μm pore size and a 300 μm internal diameter, the device can accommodate approximately 150,000 islet-like spheroids, corresponding to around 356,000 IEQs,

presenting a clinically relevant capacity for islet transplantation. Limiting the cell-cluster radius to 150 μm is intended to shorten diffusion distances and reduce the risk of hypoxia-induced central necrosis. Combined with the PES membrane, this geometry may support sustained glucose and insulin exchange while reducing structural instability associated with some hydrogel-based encapsulation systems (39).

Increasing membrane permeability may improve molecular transport and reduce hypoxia, but it can also increase graft exposure to antibodies, complement proteins, cytokines, and other soluble host factors whose effects may not be fully prevented by hypimmune engineering. Membrane design therefore involves a tradeoff between transport efficiency and residual immune exposure, rather than optimization of permeability alone (41).

Beyond this foundation, additional technologies may improve graft survival and function, but their benefits must justify the complexity and dependencies they introduce. The following subsections examine oxygen-generating biomaterials, optogenetic control, and synthetic protocells for vascular support. Each technology is first evaluated individually, then within selected HBAIS architectures to assess whether it addresses an important unresolved constraint and warrants inclusion.

7.2.3 Oxygen generation

One potential addition to the cellular and material foundation of HBAIS is a localized oxygen generating mechanism. Even with the employment of interfaces allowing for improved molecular transport, the implanted

graft remains dependent on the availability of oxygen within its surrounding environment. This is especially important immediately post-transplantation, when vascular connections with host tissue have yet to develop, leaving the transplanted graft largely dependent on diffusion for oxygen and nutrient transport (20,25).

To reduce early hypoxic necrosis, HBAIS could incorporate a temporary oxygen-generating subsystem to bridge the ischemic period before adequate host vascular connections develop. Oxygen generation must be controlled to provide sufficient support while limiting harmful accumulation of hydrogen peroxide and alkaline reaction products. OxySite, which incorporates calcium peroxide within a polydimethylsiloxane (PDMS) matrix, offers a potential approach. Water entering the matrix reacts with calcium peroxide to produce hydrogen peroxide, which subsequently decomposes to release oxygen.

Despite the benefit of sustained oxygen delivery, the oxygen generation reaction creates an important chemical constraint. Rapid exposure of calcium peroxide to water can result in rapid reaction kinetics, local changes in pH, and accumulation of potentially cytotoxic reaction intermediates (27). The hydrolysis of solid calcium peroxide must produce hydrogen peroxide first. Although hydrogen peroxide subsequently decomposes into water and oxygen gas, in situations where iron or copper ions are present, the fenton reaction occurs and releases dangerous hydroxyl free radicals (20, 27). The use of PDMS matrix has been found to control the rate of decomposition, without

substantial pH disturbance or cytotoxicity under the reported experimental conditions (42). Nevertheless, this oxygen-generating system introduces additional complexity and requires control of the calcium peroxide reaction rate to limit chemical toxicity. Its inclusion is justified only if the expected benefit of alleviating early oxygen limitation outweighs the added risks and operational dependencies.

7.2.4 Optogenetic control

Stem cell-derived β cells may exhibit less mature or slower glucose-responsive insulin secretion than native β cells (9,30). Although transcriptional optogenetic systems such as LightOn, discussed in Section 6.4, enable programmable insulin production, the delay associated with gene expression limits their suitability for rapid secretory control. GSIS also depends on oxygen-supported ATP production through oxidative phosphorylation (43). These considerations motivate an alternative optogenetic approach that amplifies the release of stored insulin through the existing GSIS pathway rather than initiating new insulin synthesis. Such amplification may improve response speed and controllability, but it does not remove the graft's requirement for adequate oxygenation.

One promising approach uses the blue-light-activated adenylyl cyclase bPAC from *Beggiatoa* to amplify GSIS. This protein couples a blue-light-sensing BLUF domain to an adenylyl cyclase domain (44). Blue-light exposure stimulates cyclic AMP production, activating downstream pathways that promote the release of stored insulin vesicles. Unlike transcriptional systems that require new insulin

synthesis, bPAC enhances secretion through the existing cellular machinery. It therefore offers rapid, externally controlled amplification while retaining the cell's intrinsic glucose-sensing mechanism, although glucose-dependent secretion must be confirmed under the intended operating conditions. bPAC converts intracellular ATP into cAMP, amplifying pathways that promote insulin exocytosis. Under the reported experimental conditions, enhanced insulin secretion was not accompanied by a measurable increase in oxygen consumption (43–44). However, bPAC does not bypass the metabolic requirements of GSIS: ATP availability, vesicle replenishment, and sustained secretion remain dependent on cellular energy metabolism.

However, bPAC depends on blue light, which is strongly absorbed and scattered by biological tissue. Fibrotic deposition around the implant may further reduce light delivery. A potential alternative is to couple bPAC with NanoLuc (nLuc), a bioluminescent enzyme that produces blue light following administration of its substrate, furimazine. Local light generation could reduce reliance on external illumination through tissue and fibrotic barriers (45). This remains a conditional strategy, requiring confirmation that substrate delivery and light output are sufficient to activate bPAC within the implanted graft. NanoLuc should be distinguished from bPAC: bPAC provides optogenetic control, whereas NanoLuc is a potential means of delivering the required optical signal. If external illumination or an implanted light source provides sufficient stimulation, adding NanoLuc would introduce

unnecessary genetic circuitry and dependence on an administered substrate.

7.2.5 EMCoac protocells

In situ oxygen-generating systems, such as calcium peroxide matrices, may provide temporary support for β -cell graft survival, while angiogenic growth factors and other tissue-engineering approaches promote vascularization. However, a mature, functional blood supply takes time to develop, and foreign-body responses to implanted materials may produce a collagen-rich fibrotic layer that separates the graft from surrounding vessels and increases resistance to molecular transport (46,47).

A key constraint on long-term graft survival is the establishment of a functional vascular supply for sustained oxygen and nutrient delivery. Immune-mediated injury and fibrotic encapsulation may further compromise graft function and separate the graft from surrounding vessels. Strategies to promote vascularization include endothelial or mesenchymal stem cell support, angiogenic growth factors, microvascular fragments, and scaffold modifications that encourage vessel growth toward the implant (15,22,46,47). A more complex candidate approach uses synthetic protocells to generate localized signals that support vascular integration.

The primary purpose of these mimics of cells are not to generate insulin, which is a task entirely done by the biological graft due to the complexity of GSIS that cannot be overcome currently by cell-free protein synthesis, but to act as a localized biochemical actuator to drive

vascularization and support grafts in addition to the oxygenation devices. Because the protocell is artificial by nature and does not depend on oxidative metabolism in the same manner as a living cell, its enzymatic function can potentially remain active in conditions which are metabolically unfavorable for biological cells. Coacervates provide a membraneless compartment that concentrates macromolecules and may enhance local reaction rates, making them a potential platform for synthetic protocells (48). Coating these structures with erythrocyte membranes may reduce immune recognition and coagulation-related responses, improve haemocompatibility, and prolong their persistence (49).

This protocell platform can compartmentalize glucose oxidase (GOx) and haemoglobin (Hb), with macromolecular crowding potentially enhancing catalytic activity; increases of up to 100-fold have been reported under the conditions examined (48). GOx converts glucose to generate hydrogen peroxide, which Hb uses in a peroxidase-like reaction involving hydroxyurea to produce nitric oxide (NO) (49).

NO activates soluble guanylyl cyclase in vascular smooth muscle cells, increasing cyclic GMP and promoting relaxation and vasodilation. NO-associated signaling can also support endothelial responses involved in angiogenesis, potentially encouraging vascular development near the implant (50). Although NO can diffuse through the surrounding extracellular environment, its effective range depends on local production, consumption, and tissue conditions; diffusion alone does not establish that it can overcome a fibrotic barrier.

GOx and Hb act catalytically rather than being consumed stoichiometrically, but they may lose activity over time. Sustained NO production therefore depends on catalyst stability and continued access to glucose, oxygen, and hydroxyurea.

However, this system is not fully self-sustaining. Continued NO production requires externally supplied hydroxyurea, creating a pharmacological dependency (49). Excessive NO can also impair mitochondrial function and cause nitrosative stress, introducing a potential cytotoxicity risk (51). The studies discussed demonstrate NO production and vasodilation,

rather than accelerated neovascularization or improved islet-graft survival. Incorporating these protocells into HBAIS therefore remains a conceptual strategy requiring evidence of benefit within the proposed graft environment.

7.2.6 Evidence-mapped constraint-transfer analysis

As established by 7.1.1., each technology is subjected to the labelling of the fixed taxonomy in isolation. Technology-level SWCE is calculated, providing the evidence map needed for later architecture-level scoring as shown in table 3.

Table 3. Summary of the constraint domains reduced and introduced by each emerging technology, their individual SWCE values, and the evidence supporting each relationship.

Intervention	Constraint domains reduced (severity)	New constraint domains introduced (severity)	SWCE	Evidence basis
Hypoimmune β -cell engineering	Immune compatibility (Critical, 3)	Tumor/genomic safety (Critical, 3), manufacturing/regulatory complexity (Minor, 1)	-1	Direct experimental evidence supports reduced immune recognition and preserved β -cell differentiation/function in relevant engineered systems; longer-term tumor/genomic safety is supported more indirectly by mechanistic and related-system evidence.
Transport-permissive retrievable interface	Long-term mass transport (Major, 2), vascular integration (Major, 2), containment/retrievability (Major, 2)	Fibrosis (Critical, 3), mechanical reliability (Major, 2)	+1	Mechanistic and related-system evidence supports improved transport, localization and retrieval. Fibrotic foreign-body responses are experimentally well established for implanted biomaterials, although their magnitude in the complete HBAIS remains untested.
Oxygen generating mechanism	Early oxygenation (Critical, 3)	Material/chemical toxicity (Major, 2), manufacturing /regulatory complexity (Minor, 1)	0	Experimental evidence supports controlled oxygen generation and the feasibility of limiting reaction-related toxicity in relevant oxygen-generating biomaterial systems; the integrated HBAIS benefit remains untested.
bPAC optogenetic control	Physiological insulin responsiveness (Major, 2)	Tumor/genomic safety (Critical, 3), external intervention dependency (Minor, 1), manufacturing /regulatory complexity (Minor, 1)	-3	Direct experimental evidence supports bPAC-mediated amplification of insulin secretion. Evidence that this additional function is required in a sufficiently mature stem-cell-derived β -cell graft is weaker.
EMCoac protocell/NO	Vascular integration (Major, 2)	Material/chemical toxicity (Major, 2), external intervention	-2	NO-mediated vascular signaling is supported by mechanistic and related-system evidence, while

vascular support		dependency (Minor, 1), manufacturing/regulatory complexity (Minor, 1)		a distinct additional benefit of EMCoac protocell within an islet HBAIS remains an architectural assumption.
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The table demonstrates that the different technologies differ in both the scope and independence of the constraints affected. Based on the calculations, the transport-permissive retrievable interface has a positive isolated SWCE under the taxonomy, whereas the other technologies yielded neutral or negative values, introducing at least as many domains as they reduce. These evidence supporting the relationship of the technology, combined with factors such as temporal role, reversibility, and observations that are considered within the nested architectural and failure-mode analyses below.

7.3. Architecture analysis of HBAIS

The technologies described in Section 7.2 can be combined into progressively more complex HBAIS architectures. The nested analysis below evaluates whether each addition addresses an unresolved constraint in the preceding configuration or transfers the burden to another domain, thereby assessing whether its benefit justifies the added complexity.

7.3.1 *Architecture I: hypoimmune stem cell-derived β cells*

The simplest architecture consists exclusively only of the hypoimmune stem cell-derived β -cell graft. This configuration provides the fundamental treatment function of restoring insulin-producing β -cell mass while reducing major pathways of host cellular immune recognition. An important advantage of this simple architecture is the absence of an encapsulating material, allowing for direct

exposure of the graft to surrounding host tissue, minimizing artificial diffusion distance and provides the most favourable conditions for surrounding vasculature and interactions to support the graft.

However, Architecture I lacks safeguards needed to protect graft survival and host safety. Hypoimmune engineering alone does not control the location, spatial distribution, or geometry of implanted cells or enable graft retrieval if abnormal behaviour develops. These limitations are particularly relevant because residual undifferentiated or partially differentiated iPSCs may retain tumorigenic potential (14). Reduced immune recognition may also weaken immune surveillance of abnormal engineered cells. Furthermore, hypoimmune engineering does not ensure complete immunological protection: antibodies, complement proteins, cytokines, and other soluble inflammatory mediators may continue to damage the graft (26,28,40,41).

Thus, although Architecture I addresses immune compatibility, tumorigenic and genomic safety remain critical concerns, while the lack of reliable physical containment introduces a major limitation. These unresolved constraints prevent Architecture I from serving as a sustainable baseline and justify evaluating additional architectures.

7.3.2 *Architecture II: hypoimmune β cells within a transport-permissive, physical interface*

Architecture II combines the hypoimmune stem cell-derived β -cell graft with a transport-permissive, retrievable physical interface. This interface localizes the graft and permits its removal if abnormal development is detected, addressing key safety limitations of Architecture I while allowing oxygen, glucose, nutrients, and insulin to diffuse across it. Control of device dimensions and graft geometry limits diffusion distances within the cellular compartment (39). If material-associated fibrosis is sufficiently minimized, the interface may also preserve proximity to host tissue and vasculature (52). Architecture II is therefore the first configuration to combine the principal permanent structural functions required for a controlled implant.

Architecture II illustrates the proposed collapse of the coupling between immune protection and restrictive transport barriers in HBAIS. If hypoimmune engineering provides sufficient cellular immune protection, the physical interface can place greater emphasis on permeability and host interaction. This upstream intervention therefore changes the design requirements for molecular transport, vascular proximity, and oxygen delivery as well as immune compatibility. The interface still introduces material-associated risks, including foreign-body responses and additional diffusion resistance relative to unencapsulated cells. Material selection and geometric optimization may reduce these risks, while containment and retrievability provide safety functions that hypoimmune engineering alone cannot supply. However, improved vascular proximity does not immediately provide vascular perfusion within the transplanted graft itself. During

implantation, the cell graft remains an avascular tissue mass supplied with nutrients through diffusion from vessels in the surrounding host tissue. Hypoimmune engineering can reduce the barrier between the host surrounding and the graft, which acts as the external oxygen source, but it is unable to instantaneously create new capillaries within or immediately to every region of the implant, nor can it increase the oxygen concentration of the surrounding tissue. During this period, lack of oxygen could still largely occur, alongside with a temporal mismatch in which the metabolic demand of the cell grafts begin immediately whereas local vascularization requires time to develop (20,25).

The controlled graft geometry attempts to reduce this limitation by shortening diffusion distance, but shorter diffusion distance alone is insufficient for adequate oxygenation. Oxygen flux also depends on the oxygen concentration gradient, membrane resistance, cellular density, and the oxygen-consumption rate of the transplanted cells. Thus, even in the absence of severe fibrosis and with substantially improved host proximity, the center of a dense graft may remain oxygen-limited (20,43). The interface therefore resolves the major containment/retrievability limitation of Architecture I and additionally benefits in terms of mass transport and vascular integration. However, its principal constraint introduced is the critical counter-risk of fibrosis, where if fibrotic deposition becomes sufficient enough, it could compromise the transport advantage that justified the permissive interface. The resulting balance therefore depends importantly on the strength of evidence that fibrosis can be adequately controlled.

7.3.3 Architecture III: Addition of oxygen generation

Architecture III supplements Architecture II with the oxygen-generating CaO_2 /PDMS mechanism described in Section 7.2.3. This localized oxygen source is intended to support the graft immediately after transplantation, before adequate host vascular support develops. Early preservation of graft mass is important because subsequent improvements in vascularization cannot simply restore cells already lost to hypoxia. Oxygen availability also supports both graft survival and glucose-stimulated insulin secretion (GSIS), as ATP production through oxidative phosphorylation is integral to glucose-responsive insulin release.

The architectural cost is an additional chemical subsystem operating close to the graft. As described in Section 7.2.3, oxygen generation may alter local pH and cause oxidative damage. Although the PDMS matrix has been shown to moderate the reaction rate and reduce adverse effects under the conditions examined, it introduces dependencies on reaction control, material integrity, and a finite reactant reservoir that are absent from Architecture II.

The oxygen source is intended to provide temporary support while host vascularization develops. Its output declines as calcium peroxide is depleted, although this depletion is not inherently synchronized with improvements in host oxygen delivery. Its finite lifetime is therefore compatible with the design if adequate vascular support develops before supplementation becomes insufficient. Chemical risks are concentrated during the active reaction period, but residual materials and

reaction products may remain relevant after oxygen generation ceases.

The architectural question for Architecture III is whether the evidence for early graft preservation through oxygen supplementation is strong enough to justify the added major material/chemical and manufacturing burdens. Its intended temporary function distinguishes this constraint exchange from a permanent operational dependency, although a finite duration does not by itself establish reversibility or eliminate residual risks.

7.3.4 Architecture IV: Addition of bPAC optogenetic control

Architecture IV integrates bPAC-mediated optogenetic control into the oxygen-supported host-integrating graft. At this stage, the principal requirements for the cell graft survival have been addressed: the β -cell population is protected from major immune-recognition pathways, physically contained for easy retrieval and control within a transport-permissive interface, and supported during the early oxygen-limited post-transplantation period. The architectural contribution of bPAC introduces an improvement to the function, rather than for survival, by rapidly amplifying insulin secretion through an externally addressable intracellular signaling pathway (44).

Successful host integration supports graft survival and function, but it may not fully resolve limitations in the intrinsic responsiveness of stem cell-derived β cells. Architecture IV adds the possibility of externally controlled amplification of glucose-

stimulated insulin secretion to the protective functions of Architecture III. This enhancement may be useful when graft cells remain less mature or responsive than native islets, provided that amplification preserves glucose-dependent secretion.

The architectural value must, however, be distinguished from necessity for survival, which remains the primary goal of the architecture. With architecture III, the cell graft is already capable of remaining viable and retaining intrinsic GSIS without bPAC. Thus, optogenetic regulation is not indispensable for graft survival or to the host-integrated β -cell replacement function.

Direct experimental evidence supports bPAC-mediated amplification of insulin secretion, but its necessity in a sufficiently mature stem cell-derived β -cell graft is less established. Its inclusion introduces a minor external-intervention dependency and may add to the existing critical tumor/genomic-safety burden through further genetic modification. bPAC is therefore considered a conditional functional enhancement rather than an essential component of the core survival architecture.

7.3.5 Architecture V: Expanded HBAIS with active vascularization

Architecture V supplements Architecture IV with the EMCoac protocell system described in Section 7.2.5 to provide active vascular support. It combines hypimmune β cells, a transport-permissive and retrievable interface, temporary calcium peroxide-based oxygen supplementation, bPAC-mediated amplification of insulin secretion, and localized nitric oxide

(NO) signaling intended to support vascular integration around the implant. Although this is the most comprehensive configuration, its justification depends on whether vascular integration remains sufficiently inadequate in the preceding architecture to warrant an additional engineered subsystem.

The host-integrating components of Architecture III are intended to support long-term oxygen and nutrient delivery by maintaining proximity to host tissue and vasculature. If adequate vascular proximity develops, EMCoac may not address a distinct unresolved constraint. Instead, it provides additional vascular signaling to reinforce a function already expected to arise through host integration.

Under implantation conditions where host-driven vascularization remains inadequate, EMCoac could provide additional vascular support. Its synthetic composition may enable localized biochemical activity without the metabolic demands of living support cells, although sustained angiogenic effects have not been demonstrated in a comparable graft environment. Inadequate vascularization alone does not establish the necessity of EMCoac, given potential NO-mediated cytotoxicity and its dependence on external hydroxyurea administration. Alternative approaches—including endothelial or mesenchymal support cells, angiogenic growth factors, microvascular fragments, and scaffold modifications—may provide vascular support with fewer added dependencies. Architecture III also provides temporary oxygen supplementation while host vascularization develops, although it cannot

substitute for adequate long-term perfusion (15,22,46,47).

Evidence for NO-mediated vascular signaling is stronger than evidence that EMCoac protocells are necessary within the graft architecture. Their principal potential benefit—improved vascular integration—overlaps with a function already credited to the host-integrating interface, reducing their marginal value when that function is adequate. EMCoac also introduces a major material/chemical toxicity concern, along with additional minor external-intervention and manufacturing dependencies.

7.3.6 Architectural synthesis

The preceding subsections of Section 7.3 examined the biological and engineering functions gained with each architecture. The architectures are now compared using the domain accounting framework described in Section 7.1.1, with each constraint domain counted once even when multiple components affect it. Marginal constraint exchanges are also calculated between successive architectures. Architecture I serves as the baseline and therefore has no marginal value; the marginal analysis begins with the transition from Architecture I to Architecture II, as shown in Table 4.

Table 4. Summary of the cumulative constraint exchange, marginal Δ SWCE, and supporting evidence across the five proposed HBAIS architectures.

Architecture	Unique constraint domains reduced	Unique constraint domains introduced	Architecture SWCE	Marginal Δ SWCE	Evidence / interpretation
I: hypimmune SC- β cells	Immune compatibility (Critical, 3)	Tumor/genomic safety (Critical, 3), manufacturing/regulatory complexity (Minor, 1)	-1	N/A	Direct evidence supports immune-evasion effects. A Critical tumor/genomic-safety concern remains without the retrieval advantage introduced in Architecture II.
II: + permissive retrievable interface	Immune compatibility (Critical, 3), long-term mass transport (Major, 2), vascular integration (Major, 2), containment/retrievability (Major, 2)	Tumor/genomic safety (Critical, 3), manufacturing/regulatory complexity (Minor, 1), fibrosis (Critical, 3); mechanical reliability (Major, 2)	0	+1	The interface improves the severity-weighted balance by adding three Major reductions while introducing one Critical and one Major constraint. Its positive marginal value reflects the combined transport, vascular-integration, and containment functions provided by a single added subsystem.
III: + Oxygen generation mechanism	Adds early oxygenation (Critical, 3)	Adds material/chemical toxicity (Major, 2), manufacturing/regulatory complexity is already represented in the preceding architecture	+1	+1	The Critical early-oxygenation constraint is reduced while the only new unique introduced domain is Major material/chemical toxicity. The manufacturing burden of the oxygen subsystem does not receive a second numerical penalty because manufacturing/regulatory

					complexity is already represented in Architecture II.
IV: + bPAC	Adds physiological insulin responsiveness (Major, 2)	Adds external intervention dependency (Minor, 1), tumor/genomic and manufacturing domains already exist upstream	+2	+1	Direct evidence supports bPAC-mediated secretory amplification. At the architecture level, the addition reduces a Major physiological-responsiveness constraint while introducing only one new unique Minor domain, producing a positive marginal SWCE.
V: + EMCoac protocell/NO	No additional unique reduced domain because vascular integration is already credited to the permissive host-integrating interface	No additional unique introduced domain because material/chemical toxicity, external intervention dependency, and manufacturing/regulatory complexity are already represented upstream	+2	0	EMCoac affects constraint domains already represented upstream and therefore provides no additional numerical constraint reduction under the fixed-domain model. Its specific benefit remains conditional: if host vascular integration proves inadequate in the provisional core configuration, the additional vascular-support function may become justified despite the present Δ SWCE of 0.

The numerical progression shows a clear pattern. The SWCE values increase from -1 in Architecture I to 0 , $+1$, and $+2$ through Architectures II–IV, where each addition of a technology to the architecture produces a marginal improvement of $+1$. Architecture V is the first addition that does not improve the severity-weighted balance, retaining the same SWCE of $+2$ and producing Δ SWCE = 0 . Thus, the analysis indicates that there is no continuous relationship between technological complexity and functional optimization. Instead, the benefit of additional subsystems starts to diminish once their targeted constraint domains are already represented in the previous configuration.

In addition, the evidence supporting the credited function differs between subsystems. The physical interface in Architecture II is supported primarily by established transport principles and biomaterial evidence, while both its localization

function and the risk of foreign-body fibrosis are well established conceptually and experimentally. For Architecture III, the benefit of oxygen generation through the hydrolysis of calcium peroxide has been demonstrated in relevant oxygen-generating biomaterial systems, supporting the proposed reduction of early oxygen limitation, although this benefit has not been validated within the complete proposed HBAIS architecture. Architecture IV has direct experimental support for the ability of bPAC to amplify insulin secretion, but the perspective that this additional amplification remains necessary in a mature graft is less established. Architecture V has similar architecture-specific justification with Architecture IV, with experimental and theoretical evidence that nitric oxide has the ability to mediate vascular signaling, but there is limited evidence that EMCoac provides an additional necessary vascular benefit once the

permissive interface is integrated to support vascular integration.

These distinctions separate the provisional core graft configuration from the additions which are functionally optional. Architecture III already contains the proposed components required to reduce immune injury, maintain physical containment and host interaction, and protect the graft during early oxygen-limited periods while preserving intrinsic GSIS.

Architecture IV provides a distinct additional function which increases SWCE by +1, but the need for this amplification in a sufficiently mature graft remains uncertain. bPAC is therefore retained as a conditional functional enhancement rather than as part of the minimum core. Architecture V similarly remains conditional, becoming justified if host-mediated vascular integration proves inadequate, even though it does not reduce a new unique constraint domain under the present architecture-level scoring.

The temporal roles of the subsystems further support this arrangement. The hypoimmune β -cell graft and physical interface form the permanent host-integrating components, while the oxygen generation mechanism is required only temporarily, providing protection during the early period before host vascular support becomes sufficient. bPAC acts as a persistent functional-control layer but is not required for basal graft survival, allowing for a rather graceful degradation should the additional bPAC mechanism fail. EMCoac, in contrast, would add another continuing subsystem

without addressing a new unique constraint under the present architecture.

Architecture III is therefore identified as the provisional core HBAIS configuration rather than selecting Architecture IV solely because it has the larger SWCE. This classification remains conditional where there is adequate control of its critical fibrosis and tumor/genomic-safety risks while maintaining reliable graft containment. Architecture IV remains as a conditional functional enhancement, and Architecture V represents a further conditional extension.

7.4 Interface compatibility analysis

A major challenge in multicomponent biological design is ensuring that the physical, chemical, and biological activities of each subsystem do not compromise the others. For HBAIS, the compatibility analysis focuses on the provisional Architecture III core and additionally considers bPAC as a conditional functional enhancement in Architecture IV. The following subsections examine potential interactions that could impair graft safety or function.

7.4.1. *Localized oxygen-generating chemistry & beta cell tolerances*

The hydrolysis of solid calcium peroxide within HBAIS creating oxygen gas is used to prevent core necrosis, but must produce hydrogen peroxide first. Although hydrogen peroxide decomposes into water and oxygen gas, in situations where iron or copper ions are present, the fenton reaction occurs and releases dangerous hydroxyl free radicals (20, 27). To prevent the oxygen generation mechanism

interfering with the well-being of the cell grafts, HBAIS places the oxygen generating calcium peroxide inside a highly hydrophobic polydimethylsiloxane membrane, where the entire oxygen generation subsystem is further placed within the PES membrane.

The hydrophobic membrane severely restricts water infiltration. Water influx across the PDMS membrane is approximated using Fick's first law of diffusion; this influx influences calcium peroxide hydrolysis:

$J = -D_{\text{water, PDMS}} \frac{\partial [H_2O]}{\partial x}$, where D_{water} is the diffusion coefficient of water within the PDMS matrix, while $\frac{\partial c}{\partial x}$ represents the concentration gradient of water (53). In the PDMS membrane, the water diffusion coefficient is low, $\sim 10^{-9} \text{ m}^2/\text{s}$ (54). Restricted water ingress can help limit hydrolysis, while oxygen diffuses outward through PDMS, with a reported diffusion coefficient of $(3.55 \times 10^{-9}) \text{ m}^2/\text{s}$ (55). However, outward oxygen transport alone does not establish that hydrogen peroxide remains below cytotoxic concentrations; peroxide exposure also depends on its formation, decomposition, and transport. OxySite experiments reported peroxide concentrations below cytotoxic thresholds under the conditions examined, supporting the feasibility of controlled oxygen generation (42). These findings provide a basis for the proposed design, but Fickian transport alone cannot predict local peroxide exposure within the complete HBAIS, which requires further validation.

7.4.2 Fibrotic buildup effects on optogenetic control

Implanted biomaterials can trigger a foreign-body response that forms a dense, collagen-rich fibrotic capsule around the graft, increasing resistance to molecular diffusion. This capsule may also attenuate and scatter incoming light, reducing optical stimulation and impairing optogenetic control.

Many optogenetic actuators respond to blue light, typically around 450–485 nm, whose penetration is limited by tissue absorption and scattering (30). Fibrotic tissue surrounding an HBAIS graft could further attenuate illumination, reducing the effectiveness of externally controlled insulin secretion. Red and near-infrared light generally penetrate tissue more deeply, but cannot directly substitute for blue light when using the blue-light-sensitive actuator bPAC.

One potential approach is to couple bPAC with NanoLuc, a luciferase that produces blue bioluminescence in the presence of its substrate, furimazine. Local light production could reduce reliance on illumination through overlying tissue (45). However, effective activation would depend on sufficient light output, appropriate coupling to bPAC, and substrate delivery. This strategy has not been demonstrated in the proposed implanted β -cell graft under fibrotic conditions. NanoLuc therefore remains a conditional optical-delivery option, whose benefits must justify the additional genetic circuitry and substrate dependency, rather than a routine component of Architecture IV.

7.4.3 Hypoimmune genomic edits effects on long-term functional maturation & stability

Hypoimmune engineering through B2M and

CIITA knockout raises concerns about possible effects on the transcriptional, metabolic, and functional maturation of stem cell-derived β cells, including glucose sensing and insulin secretion *in vivo* (28). Nevertheless, human iPSCs carrying these knockouts together with CD47 overexpression retained the capacity to differentiate into pancreatic islet cells. Following a reported 22-day differentiation protocol, wild-type and hypimmune iPSCs produced comparable proportions of insulin-producing β cells and glucagon-producing endocrine cells, with no significant reduction in differentiation capacity under the conditions examined.

Furthermore, wild-type, double-knockout, and hypimmune islets exhibited comparable insulin secretion. Under the reported experimental conditions, hypimmune modifications did not measurably impair differentiation or insulin secretion relative to the controls (56). These findings support the preservation of β -cell function following hypimmune engineering, although they do not establish long-term functional stability or genomic safety.

Together, the retained interfaces within the architecture illustrate that compatibility within the provisional Architecture III core is plausible although not fully proven, while the additional bPAC mechanism becomes relevant if the Architecture IV enhancement is adopted. Successful integration still depends on controlling the rate of oxygen generation, preservation of β -cell function with hypimmune editing, and maintenance and selection for the most optimal transport through the physical interface, with sufficient optical access representing an additional requirement only when bPAC is included.

7.5 Failure modes and reversibility of HBAIS

The provisional Architecture III core has several potential failure modes, while optional components such as bPAC and EMCoac introduce additional risks. System robustness depends on the consequences of component failure and the dependencies introduced by any backup mechanism. A failure may permit graceful degradation, in which an added capability is lost but essential graft functions remain intact, or it may irreversibly compromise graft survival or containment. Table 5 evaluates these potential failures and their implications for HBAIS safety and function.

Table 5. Analysis of potential failures and their effects on the HBAIS system

Failure mode	Consequence for HBAIS	Severity & reversibility	What this reveals about the HBAIS architecture
Incomplete hypimmune protection	Residual cellular and humoral immune attack progressively damages the transplanted β -cell population, reducing viable graft mass and insulin output.	Progressive and irreversible. Lost β -cell mass cannot be restored by the remaining subsystems.	The host-integration strategy relies heavily on hypimmune engineering reducing immune injury sufficiently to permit a permissive interface. This is therefore a condition of the entire architecture, rather than a failure that another subsystem can

			readily compensate for.
Progressive fibrosis around the physical interface	Increasing diffusion distance gradually restricts oxygen, nutrient, glucose, and insulin exchange even though the cells and device remain physically intact.	Progressive and potentially partially reversible only if the foreign-body response can be controlled.	This failure directly attacks the mechanism responsible for close host interaction. It therefore represents the main long-term threat to the host-integration advantage of the provisional Architecture III configuration.
Physical rupture or loss of graft containment	Engineered cells may escape the controlled compartment, eliminating reliable localization and retrieval of the graft.	Acute and potentially catastrophic. Once cells escape, removal of the intact device may no longer remove the complete graft.	This consequence is distinct from the Major mechanical-reliability constraint in Table 2, which represents performance-reducing structural degradation while containment remains intact. Once structural failure produces loss of containment and possible escape of engineered cells, it becomes a catastrophic safety failure rather than an ordinary mechanical-reliability limitation.
Insufficient oxygen support during early engraftment	β cells may experience severe hypoxia before host vascular support has developed, causing early loss of implanted cell mass and weakening subsequent graft function.	Phase-specific but potentially irreversible. The vulnerability is highest early after implantation and decreases once host oxygen delivery becomes sufficient.	This is a timing and supply failure rather than a permanent architectural deficiency. It reinforces why temporary oxygen support is retained despite its chemical complexity, as the host-integrating architecture cannot compensate quickly enough during this early window.
Failure of bPAC expression or optical activation	Externally addressable amplification of insulin secretion is lost, but intrinsic GSIS and the underlying cell graft remain functional.	Generally non-catastrophic and functionally reversible if optical activation can be restored.	This represents graceful degradation of the conditional Architecture IV enhancement. Loss of bPAC removes an additional secretory-control capability without abolishing the intrinsic GSIS or survival functions already present in Architecture III.
Inadequate long-term host vascularization	After temporary oxygen support declines, insufficient vascular proximity may produce persistent oxygen and nutrient limitation, progressively impairing β -cell survival and GSIS.	Progressive and potentially severe, but generally slower than early oxygen failure.	This defines an operating boundary of Architecture III. If host-mediated vascular integration remains insufficient after the temporary oxygen-support period, an additional vascular-support strategy may become justified, including the EMCoac/NO subsystem represented by Architecture V.

If mitigation of a failure requires the HBAIS. The consequences of structural failure incorporation of another subsystem, this is also depend on its extent. For example, treated as a compensatory complexity rather mechanical degradation may progressively than automatic justification for expansion of impair device performance and is therefore

classified as a major reliability constraint. If deterioration progresses to complete rupture, however, engineered cells may escape the controlled compartment, eliminating reliable localization and retrievability and produce a potentially catastrophic safety failure. Long-term mechanical integrity and containment must therefore be maintained throughout implantation.

Incomplete immune protection threatens the biological premise of safe host integration, fibrosis progressively undermines the transport advantage created by the permissive interface, and membrane rupture leads to destructive safety failure. Insufficient early oxygenation is instead a phase-specific vulnerability, whereas inadequate long-term vascularization defines the potential for additional subsystem introduction, representing the biological operating boundary of the design. In contrast, failure of bPAC produces graceful degradation as intrinsic GSIS function remains intact. The failure mode analysis therefore distinguishes vulnerabilities that threaten the provisional Architecture III core from failures of optional additional subsystems. HBAIS robustness requires prioritizing the prevention of failures that irreversibly compromise graft survival or containment, while adding backup mechanisms only when their benefits justify the additional complexity.

7.6 Assumptions adopted In HBAIS analysis

The HBAIS analysis examines graft dynamics, metabolic viability, and interface safety by considering interactions among subsystems and the conditions that limit their compatibility. It draws on biological, chemical, and physical

principles to assess conceptual feasibility before *in vivo* testing. The following assumptions define the scope and limitations of this qualitative and first-order analysis.

7.6.1 Restoration of β -cell mass as the primary pathological target

The biological modeling of glycemic control assumed that the implanted stem-cell-derived clusters are able to function compatibly with native host islet cells. This assumption was used because the cause of T1DM can be mainly attributed to autoimmune destruction of insulin-producing pancreatic β -cells, meaning that directly replenishing the insulin-secreting mass represents the most physiologically convincing pathway to achieve insulin independence. If restoring β -cell mass alone is insufficient—for example, because inadequate paracrine signaling from glucagon-producing α cells or somatostatin-producing δ cells impairs glucose-stimulated insulin secretion—the graft may fail to achieve adequate glycemic control (9). This failure could potentially create insulin-induced hypoglycemia, leading to severe consequences. Nevertheless, clinical outcomes following the Edmonton Protocol support β -cell replacement as a means of improving glycemic control and reducing or eliminating exogenous insulin requirements in selected patients with T1DM (12).

7.6.2 Size-selective physical containment and genetic immunoevasion

The analysis assumes that a transport-permissive polyethersulfone (PES) membrane, combined with genetically engineered cell clusters, can maintain physical containment while reducing reliance on restrictive

immunoisolation. Pores smaller than host immune cells but sufficiently large to permit molecular exchange may facilitate nutrient and glucose transport. However, antibodies, cytokines, and complement proteins can still cross the membrane and potentially damage the graft. The proposed configuration therefore assumes that residual exposure to these soluble immune factors remains compatible with graft survival. If this assumption fails, additional immune protection or a more restrictive membrane may be required, potentially reintroducing transport limitations and hypoxia (39,40).

The architectures therefore assume that the remaining and possible humoral exposure does not produce sufficient harm to outweigh the transport advantages gained from the more permissive host-integrating interface. If this assumption is invalid, the design may require greater physical exclusion or additional immune protection, in turn reintroducing the diffusion and hypoxia constraints that the architecture strives to reduce.

7.6.3 Fickian transport and oxygen generating kinetics

The transport analysis assumes that oxygen generation produces an approximately steady flux that can be described by one-dimensional Fickian diffusion across the polymer matrix (27). Because the PDMS layer is assumed to be thin relative to the graft radius, a locally planar approximation is used to simplify calculations of water influx and oxygen transport. This approximation neglects curvature and spatial variation within the graft and therefore provides

only a first-order assessment of transport feasibility.

If this assumption fails, variations in water influx may alter hydrolysis kinetics. Excessive influx could accelerate reactant depletion, exhausting oxygen support before adequate host vascularization develops, while insufficient influx could limit oxygen production. Inadequately controlled reactions may also cause local accumulation of hydrogen peroxide and alkaline byproducts, potentially damaging graft cells. These effects could compromise graft survival through hypoxia, chemical toxicity, or both.

7.6.4 Glucose-controlled optogenetic control mechanism

The optogenetic mechanism assumes that bPAC primarily amplifies glucose-stimulated insulin secretion (GSIS) and that the absence of a measurable increase in oxygen consumption reported experimentally also applies within the proposed graft. Upon light activation, bPAC converts intracellular ATP into cyclic AMP (cAMP), enhancing signaling that promotes exocytosis of stored insulin vesicles rather than initiating new insulin synthesis. This amplification is assumed to remain dependent on glucose-responsive signaling and calcium-dependent secretion (44). If glucose dependence is insufficiently preserved, optical stimulation could provoke inappropriate insulin release at normal or low blood glucose levels, increasing hypoglycemia risk. Sustained stimulation could also place demands on cellular energy metabolism and insulin-vesicle replenishment. The integrated system therefore requires

validation of glucose dependence, metabolic tolerance, and sustained secretory capacity.

7.6.5 Limitations of the constraint exchange framework

The mathematical model utilized throughout the analysis, SWCE framework, is intended as a heuristic method for making transparent architectural reasoning rather than to be used as a confirmed predictor of biological and clinical performance. The fixed weights assigned to minor, major, and critical constraints provide a transparent scoring convention for distinguishing their consequences. However, constraints within the same category may affect graft survival differently, and the numerical weights do not represent a validated linear scale of biological severity.

The methodology of assigning a fixed weight to each constraint domain and accounted only once within an architecture, is employed to prevent compounding scoring, but may simplify situations in which multiple mechanisms might exacerbate the same underlying problem.

The immune-compatibility credit assigned to hypoimmune engineering would be weakened if edited grafts exhibited immune-mediated loss comparable to unedited controls. The transport and vascular-integration credits assigned to the permissive interface would likewise be weakened if it failed to improve molecular transport or vascular proximity, or if fibrosis negated these benefits. The early-oxygenation credit assigned to calcium peroxide would be unsupported if oxygen supplementation failed to improve early graft survival compared with the same configuration without supplementation.

The physiological-responsiveness credit assigned to bPAC would be unnecessary if mature stem cell-derived β cells achieved adequate glucose-responsive insulin secretion without amplification. Finally, EMCoac would gain functional justification if vascular integration remained inadequate and its addition measurably corrected that deficiency, even if the fixed-domain score did not change.

SWCE scores should be interpreted alongside the strength of evidence supporting each assigned benefit or burden. New experimental findings may require reassignment of the domains reduced or introduced by a technology, even if the predefined severity weights remain unchanged. These revisions may alter the scores and the resulting architectural preferences.

8. Challenges

8.1. Efficacy and safety of emerging technologies

Although emerging technologies offer promising approaches to β -cell replacement in T1DM, their clinical translation remains constrained by unresolved safety, efficacy, and manufacturing challenges. The proposed HBAIS likewise requires validation as an integrated system before clinical application.

[1] Genomic complexity and off-target risks: Individual immune-evasion modifications may provide incomplete protection. Gaykema et al. found that B2M knockout alone did not prevent T-cell-mediated rejection of iPSC-derived kidney organoids; this finding concerns the studied organoid model and does not directly establish outcomes for β -cell grafts (41).

Combining modifications may broaden immune protection, but increases engineering and quality-control demands. Gene editing can also introduce unintended genomic changes (26). These concerns are particularly relevant to Architecture IV, which combines hypimmune modifications with optogenetic circuitry. Future development should therefore identify the minimum modifications required for adequate immune protection and functional control.

[2] The mixed characteristics of immune engineering: The development of hypimmune cells via HLA gene can benefit by becoming “invisible” and resistant to immune detections, yet can also inadvertently create an opportunity for growth of tumors and malignancies. Without immune surveillance that keep cancerous cells from developing, mutations in these hypimmune cells could go unchecked if they do arise. Hypimmune engineering should therefore be viewed as a method for reducing selected pathways of immune rejection rather than making the graft completely invisible or universally compatible with the host. Long-term studies will be required to determine whether sufficient immune protection can be achieved without creating unacceptable risks from reduced surveillance or residual humoral immunity.

[3] Performance of safety switches: Inducible suicide-gene systems offer a potential means of eliminating engineered cells if abnormal growth or other complications arise. However, incomplete cell elimination can leave surviving cells capable of further proliferation, so these mechanisms should not be assumed to provide complete protection against tumor formation

(26). The proposed HBAIS therefore prioritizes physical containment and retrievability as its primary safety features. Genetic kill switches may provide additional protection, subject to validation of their effectiveness, stability, and safety in the relevant β -cell graft context.

Future research should examine whether immune protection can be achieved with fewer genetic modifications or through complementary strategies that reduce the need for additional edits. Antibody-based approaches and engineered immune-cell strategies warrant investigation, but their mechanisms and effectiveness must be established in the relevant β -cell graft context (28). A key translational milestone is sustained glycemic control following islet transplantation without systemic immunosuppression, accompanied by evidence of graft survival, glucose-responsive insulin secretion, and acceptable long-term safety. These outcomes would provide a stronger basis for evaluating clinical feasibility.

8.2 Overcoming the foreign body response

Despite innovations such as oxygen-generating technologies and hypimmune cell technologies, immediate graft loss remains substantial. Historical data indicate that less than 60% of transplanted islets successfully engraft, with a significant proportion lost due to the immediate blood-mediated inflammatory reaction (IBMIR) (47). Even with “stealth” materials, the host may form a dense fibrous capsule that chokes off nutrient supply and increase distance between the graft and surrounding vasculature. Future research should therefore prioritize biomaterials and surface modifications that reduce long-term fibrotic

deposition while maintaining permeability, mechanical durability, and retrievability. For example, alginate modified with zwitterionic functional groups has shown to substantially reduce material overgrowth around grafts (52).

8.3 Manufacturing and metabolic scaling

Another significant barrier currently still persisting is the high volume of cells required. Clinical islet transplantation requires substantial cell quantities, with insulin independence depending on graft quality, transplanted islet mass, recipient characteristics, and post-transplantation survival (9). For patient-specific iPSC-derived grafts, individual HLA profiles and the need for customized cell production and quality control increase manufacturing complexity. Maintaining cell purity, functional consistency, and viability during large-scale production remains a major barrier to widespread clinical use.

Standardized master cell banks offer a potential strategy for reducing manufacturing complexity. These banks contain thoroughly characterized cell lines selected for HLA matching or engineered to reduce immune recognition, potentially serving multiple recipients without requiring patient-specific iPSC production. Establishing such lines allows manufacturers to optimize reproducible differentiation, expansion, and quality-control processes (26). However, broad applicability depends on demonstrating genetic stability, consistent β -cell function, and adequate immune compatibility; hypimmune engineering does not guarantee universal protection from rejection.

9. Perspectives: exploring decoy strategies for islet graft immune protection

An alternative to increasing graft isolation or hypimmune engineering is to investigate whether an auxiliary implant can selectively intercept graft-reactive immune cells or antibodies. Jacoby and Brock proposed antigenically identical decoy tissue for this purpose, with the additional possibility of promoting donor-specific immune suppression (57). Clinical experience with simultaneous islet–kidney transplantation demonstrates that multiple grafts can coexist while providing sustained glycemic benefit (58). This observation motivates investigation of whether an auxiliary, antigenically matched graft could also reduce immune injury to a principal islet implant. However, Lehmann et al. did not test immune diversion, and their findings do not establish that the kidney graft acted as a decoy (58). Applied to HBAIS, this concept would transfer part of the protective burden to an auxiliary site while the principal implant establishes vascular connections. Immune diversion and durable tolerance are distinct outcomes, however, and neither can be assumed from the presence of a second graft. Furthermore, these approaches remain exploratory and are considered potential future extensions of HBAIS rather than components of the provisional core architecture.

9.1 Lymph-node implantation

Donor-antigen-matched islets placed within a lymph node could potentially combine insulin production with interception or regulation of graft-reactive immune responses. In a syngeneic diabetic mouse model, islets transplanted into the jejunal lymph node engrafted and restored

normoglycemia (59). However, although these findings support the lymph node as a viable transplantation site, they have yet to demonstrate immune diversion, donor-specific tolerance, or protection of a separate graft. The proposed extension is therefore to determine whether lymph-node implantation reduces immune injury elsewhere or instead enhances antigen presentation and rejection.

9.2 Retrievable decoy implants

A separate, retrievable implant containing relevant donor antigens could provide a more controlled application of the original decoy concept (57). Its intended function would be to capture graft-reactive effectors or encourage donor-specific regulation while preserving the principal graft. Retrievability would permit removal if inflammation or sensitization develops. The central uncertainty is whether the decoy reduces destructive immune activity rather than amplifying it. Another consideration is whether the decoy presents antigens relevant to the hypoimmune-engineered primary graft, whose antigen-expression profile may differ from the unmodified donor tissue. Inadequate matching or excessive antigen presentation could potentially increase immune sensitization instead of enhancing protection.

9.3 Temporary auxiliary intraportal islet grafts

An auxiliary intraportal islet graft could be administered alongside the principal implant, providing additional insulin secretion while potentially serving as an antigenically matched decoy. Lantidra establishes the clinical feasibility of intraportal donor-islet infusion, but does not demonstrate immune diversion or tolerance induction; the auxiliary intraportal

graft would also lack the principal implant's retrievability (60). The appropriate duration of auxiliary graft exposure would require experimental determination, including whether any observed immunological benefit persists after the auxiliary intervention is withdrawn. Additional donor exposure, procedural risks, and immunosuppression requirements would need to be weighed against any protective benefit. Successful neovascularization would improve metabolic support but would not itself establish immune tolerance or justify withdrawal of immunosuppression.

Experimental evaluation would determine whether these decoy strategies warrant incorporation as optional modules within the HBAIS heuristic framework, based on demonstrated reductions in graft injury balanced against sensitization risks, procedural burdens, and operational dependencies. This illustrates an additional advantage of the framework: its modular structure can accommodate newly identified technologies and mechanisms as research advances, using the same criteria to assess whether their benefits justify the burdens they introduce.

10. Conclusion

Durable β -cell replacement in T1DM requires coordinated management of immune compatibility, molecular transport, vascular support, functional responsiveness, and graft safety. This review proposes a heuristic constraint-exchange framework to examine whether each technological addition addresses an unresolved limitation or transfers burden to another domain. Its contribution lies in making architectural assumptions and tradeoffs explicit,

including the diminishing value of overlapping functions and the dependencies introduced by additional subsystems.

Applied to the proposed hybrid bio-artificial islet system (HBAIS), the analysis identifies hypimmune stem cell-derived β cells within a retrievable, transport-permissive interface, supplemented by temporary oxygen generation, as a provisional core configuration. Optogenetic amplification and synthetic protocell-mediated vascular support remain conditional additions when intrinsic glucose responsiveness or host vascular integration is inadequate. Component selection therefore prioritizes essential survival and safety functions before introducing further complexity.

Auxiliary donor-antigen-matched decoys offer a complementary hypothesis: immune interactions might be intercepted or regulated at a separate site to reduce injury to the principal graft. Lymph-node grafts, retrievable decoy

implants, and auxiliary intraportal islets warrant evaluation for this purpose. Their incorporation as optional HBAIS modules would depend on demonstrated protection rather than increased sensitization, with temporary immune diversion distinguished from durable tolerance.

The framework remains heuristic, and the integrated architectures are experimentally unvalidated. Future work should establish whether these strategies produce sustained functional benefit without unacceptable fibrosis, chemical toxicity, genomic risk, or external dependencies. Such evidence would determine which combinations justify their complexity and support more durable β -cell replacement.

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