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Islet Transplantation: Microencapsulation, Nanoencapsulation, and Hypoimmune Engineering

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ABSTRACT

Islet transplantation represents a promising curative approach for type 1 diabetes by restoring glucose-responsive insulin secretion. However, the requirement for lifelong immunosuppression to prevent immune rejection can lead to significant side effects. Emerging strategies such as microencapsulation, nanoencapsulation, and hypoimmune engineering are being developed to protect transplanted islets from immune attack, thereby enhancing their viability and function. This review critically examines these innovative technologies, highlighting the methodologies, materials, experimental and clinical outcomes, as well as the challenges they face and potential solutions to overcome those challenges.

1 | Introduction

Diabetes mellitus is a chronic metabolic disorder characterized by impaired glucose homeostasis (Cole and Florez 2020; Nathan 1993). The disease manifests in two major forms: type 1 diabetes (T1D) and type 2 diabetes (T2D). T1D is an autoimmune disease. It is characterized by the selective destruction of insulin-producing pancreatic β-cells, which leads to absolute insulin deficiency (Katsarou et al. 2017). T2D, marked by insulin resistance and progressive β-cell dysfunction, accounts for more than 90% of diabetes cases in adults (Chatterjee et al. 2017). T1D and T2D impose a significant global burden, affecting over 537 million people and contributing to severe complications such as cardiovascular disease, kidney failure, and neuropathy (International Diabetes Federation 2021). Despite advances in pharmacological management, the quest for curative and long-term therapies remains a key focus, particularly for T1D patients reliant on exogenous insulin.

In T1D, autoreactive T cells fail to recognize β-cells as self (Katsarou et al. 2017). Resultantly, the immune system targets and destroys β-cells in the pancreatic islets of Langerhans. It is further exacerbated by pro-inflammatory cytokines and innate immune activation, which result in chronic hyperglycemia and associated metabolic dysregulation. T1D requires lifelong insulin replacement therapy. However, exogenous insulin cannot replicate the precise physiological regulation of endogenous insulin secretion, which leads to frequent episodes of hypoglycemia or hyperglycemia. For many T1D patients, particularly those with brittle diabetes or hypoglycemia unawareness, these challenges highlight the need for more effective therapeutic strategies to restore glycemic control and preserve residual β-cell function.

Islet transplantation represents a promising curative approach for T1D, as transplanted islets can, in principle, restore glucose-responsive insulin secretion and ameliorate the burden of

TABLE 1 | Advantages and disadvantages of microencapsulation, nanoencapsulation, and hypoimmune engineering.

Method	Advantages	Disadvantages
Microencapsulation	– Provides immunoisolation by forming a permselective barrier – Controllable microcapsule size. – Uniform microcapsules – Blocking immune cells – Avoiding lifelong immunosuppression – Suitable for allogeneic and xenogeneic islets or islet-like cell aggregates	– Mostly requiring instrumentation – Involving harsh conditions – Fibrotic overgrowth. – Limited molecular transport – Empty microcapsule production – Large transplantation volume
Nanoencapsulation	– Instrument-free manufacturing – Reducing graft volume – Allowing easy oxygen, nutrient, and waste diffusion – Allowing tunable surface functionalization with bioactive molecules or nanoparticles	– Insufficient protection – Degradation of nanocoating – Iterative operation – Cell damage – No clinical testing
Hypoimmune engineering	– Encapsulation-free production – Direct vascularization – Defined surface modification – No immunosuppression – Reduced inflammatory response – Potential for personalized medicine	– Genetic modifications – Requiring extensive validation and safety control – Insufficient protection – Regulatory challenges

exogenous insulin dependence (Shapiro et al. 2017). However, the immune system's attack on transplanted islets remains a significant barrier to the success of this therapy. Therefore, systemic immunosuppression has to be used in current transplantation protocols. The first cellular therapy for T1D patients was approved by the Food and Drug Administration (FDA) on June 28, 2023 (U.S. Food and Drug Administration 2023). Lantidra, marking a groundbreaking milestone in diabetes treatment, utilizes allogeneic islet transplantation to restore glucose-responsive insulin secretion in eligible patients, particularly those with hypoglycemia unawareness or severe glycemic lability. However, the treatment requires lifelong immunosuppression to prevent graft rejection, which poses risks such as infection, malignancy, and organ toxicity. VX-264, an investigational islet cell therapy encapsulated in a proprietary macroencapsulation device developed by Vertex, completed Phase 1/2 dosing. However, the analysis did not meet its efficacy endpoint, resulting in the termination of the clinical trial (Vertex Pharmaceuticals Inc. 2025). Therefore, great efforts have been made in developing innovative strategies to achieve immune tolerance or isolate transplanted islets from immune attack.

Encapsulation is a technology of enclosing living cells with a semi-permeable membrane (Desai and Shea 2017; Liu et al. 2025). As cells are shielded behind the membrane from being recognized by immune cells and antibodies, cell encapsulation leads to effective immune isolation. Meanwhile, due to a small size or short diffusion distance, the membrane allows the diffusion of oxygen, nutrients, and insulin. The semi-permeable microcapsule concept originated from Chang's artificial cell system. The system was further advanced by Lim and Sun, who pioneered the microencapsulation of islets, creating the first bioartificial endocrine pancreas (Chang 1964; Lim and Sun 1980; O'Shea et al. 1984). Two main encapsulation approaches have been widely studied: microencapsulation and nanoencapsulation. Microencapsulation refers to a spherical system ranging in size from approximately tens of microns to 1.5 mm. Nanoencapsulation, by contrast, refers to nanometer-scale coatings or layers directly deposited on the islet surface. Both encapsulation methods aim to reduce immune rejection and eliminate the need for systemic immunosuppression, offering a promising path to improved islet viability and functionality in T1D treatment. As this review focuses on small-scale technologies, macroencapsulation or device-related islet transplantation will not be discussed in this review. Interested readers can find information from other relevant review articles (Desai and Shea 2017; Liu et al. 2025).

Hypoimmune engineering offers an alternative approach for islet transplantation. It is a new frontier in the field of islet transplantation. By genetically modifying donor islets or their source tissues to evade immune recognition, hypoimmune engineering seeks to create “invisible” grafts that are inherently resistant to immune attacks. This approach is facilitated by advances in gene editing technologies, such as CRISPR-Cas9, which enable the precise alteration of immune-related pathways to diminish graft immunogenicity (Hotta et al. 2024). Hypoimmune engineering has the potential to redefine the therapeutic landscape of cell therapy, such as islet transplantation.

In this review, we provide a concise overview of microencapsulation, nanoencapsulation, and hypoimmune engineering. We discuss the methods, materials, and both experimental and clinical outcomes associated with these approaches, as well as the challenges they face. The advantages and shortcomings of microencapsulation, nanoencapsulation, and hypoimmune engineering are summarized in Table 1.

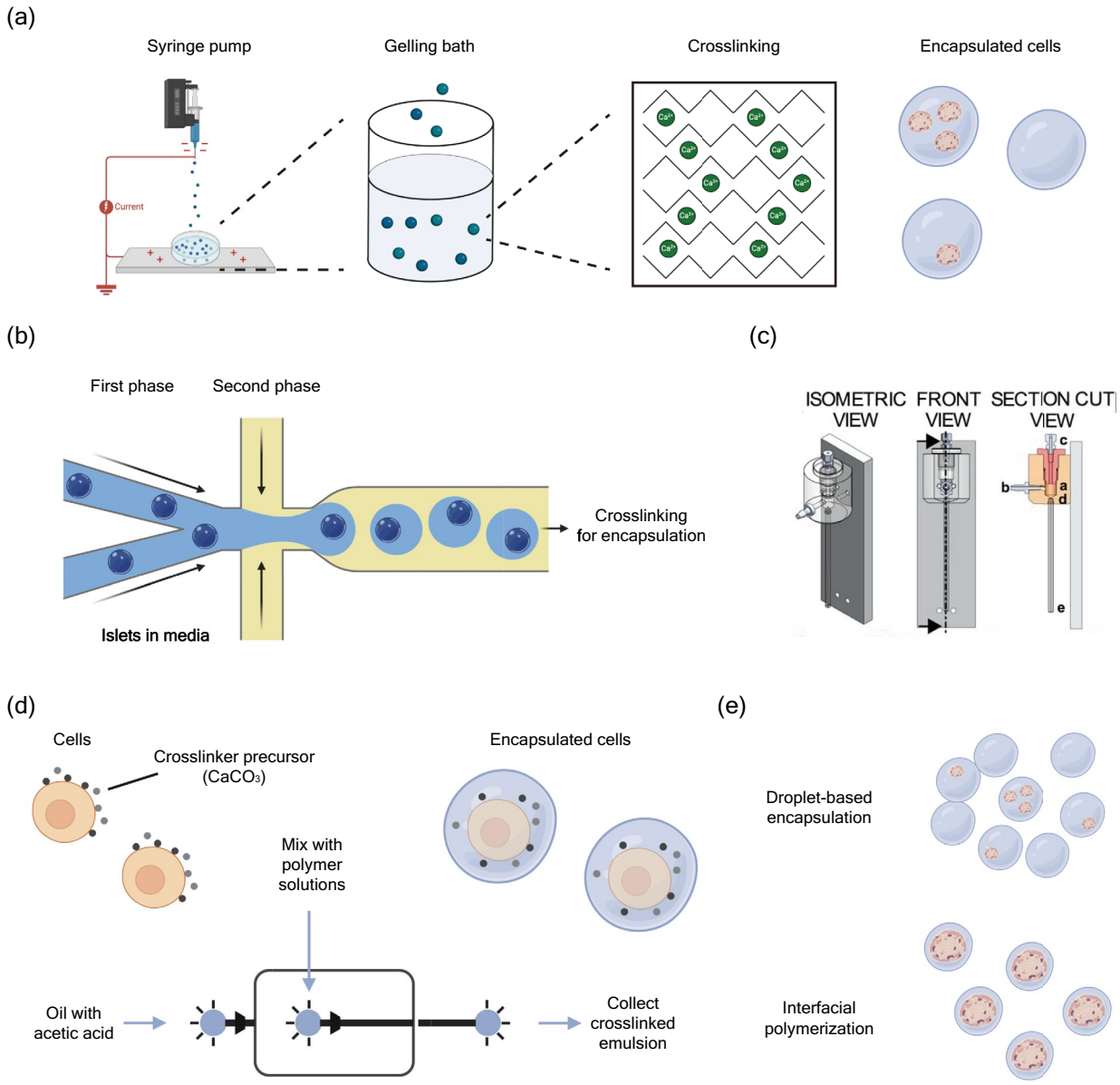

FIGURE 1 | Methods for islet microencapsulation. (a) Syringe pump-based droplet generation. An alginate solution with the cell suspension is extruded from the syringe and crosslinked by bivalent cations. (b) Microfluidics-based droplet generation. The aqueous solution with the cell suspension forms droplets in an organic phase. (c) Optimized microfluidic device. Adapted from Tomei et al. (2014). (d) Interfacial polymerization. The adsorbed $CaCO_3$ on the cell surface is released by acetic acid, and the released Ca ions induce alginate crosslinking. Adapted from Mao et al. (2017). (e) Comparison of droplet formation and interfacial polymerization in cell microencapsulation: The former method is associated with the formation of empty microcapsules and a large polymer volume, whereas the latter achieves nearly 100% encapsulation efficiency and uses a much smaller polymer volume.

2 | Microencapsulation

Thomas Chang described a method for encapsulating bioactive enzymes using semi-permeable microcapsules in 1964 and briefly mentioned the potential for applying this approach to cell encapsulation (Chang 1964). However, it was not until 1980 that Lim and Sun experimentally explored islet microencapsulation within an alginate-polylysine-alginate membrane (Lim and Sun 1980). Since then, microencapsulation has advanced significantly, with innovations in methods, materials, and applications in transplantation. The historical development and foundational breakthroughs in this field are introduced below to trace its evolution.

2.1 | Methods

2.1.1 | Liquid Extrusion From Syringe

Lim and Sun demonstrated the first microencapsulation of islets as a bioartificial endocrine pancreas using the liquid extrusion method in 1980 (Lim and Sun 1980). In their study, islets

were suspended in an alginate solution (Figure 1a). The islet suspension was extruded from a syringe needle to generate cell-containing alginate droplets. The droplets were immediately solidified to form spherical alginate hydrogel microparticles once reaching the $CaCl_2$ solution. The microparticles were further reacted with poly-L-lysine and alginate sequentially to form alginate-poly-L-lysine-alginate microcapsules.

Early efforts were focused on developing microcapsules with diameters of several hundred microns, as it was hypothesized that thin microcapsules would benefit cell survival compared to larger microcapsules. This hypothesis on designing thinner microcapsules seems reasonable from the viewpoint of molecular transport, as the permeability of a microcapsule is inversely related to its thickness of molecular transport. A shorter distance diffusion leads to a higher permeability of insulin, nutrients, oxygen, and wastes, which is beneficial to support the survival of encapsulated islets. However, Veiseh et al. recently demonstrated that microcapsules with a large size could prolong in vivo cell survival for treating immunocompetent diabetic mice (Veiseh et al. 2015). Ba^{2+}-crosslinked alginate microcapsules were fabricated in different diameters. The researchers employed precise control of extrusion parameters and crosslinking conditions to acquire different diameters of alginate microcapsules. Adjustments in nozzle diameter, extrusion speed, and crosslinking time allowed for consistent and reproducible fabrication of hydrogel spheres with narrowly distributed sizes. While larger microcapsules were found to allow for a longer period of glycemic control compared to smaller ones due to the fact that larger microcapsules reduced fibrosis and immune reactions, this method did not address whether the larger diffusion distance would not be an issue to molecular transport for the reasons discussed above and what portion of encapsulated islets could survive and function during the long-term transplantation. Potential challenges, such as lack of adequate oxygen and nutrient diffusion may remain a concern for long-term diabetes treatment.

Another question related to the large diameter is the transplantation volume. While a larger microcapsule would have the capability of encapsulating more than one islet cluster, it is possible that the polymer-to-cell ratio for a larger microcapsule may be greater than that for a smaller one. If this case holds true, using microcapsules with larger diameters would lead to an increased transplantation volume. Given that a human adult patient needs one million islets and one islet is 150 μm in diameter, the transplanted islets would have 1.8 mL in volume. When these islets are encapsulated in 500 μm microcapsules, the final volume would be 65.4 mL. If a 1.5 mm microcapsule could encapsulate three islets, the final transplantation volume would be 589 mL. This simple calculation suggests that using a large microcapsule would be associated with a large transplantation volume. It is also important to note that the process of encapsulating islets based on liquid extrusion is inevitably accompanied by the formation of empty microcapsules or microcapsules with a small number of islets.

Considering the molecular transport issue, a following study from the same group studied the effects of cell cluster size on cell transplantation in addition to the size of microcapsules (Bochenek et al. 2024). Smaller human stem cell-derived β-cell clusters (SC-β; ~150 μm) encapsulated in 1.5 mm alginate microcapsules demonstrated better glycemic control and reduced fibrosis compared to larger clusters (~300 μm). This positive result indicates that resizing or reducing the size of cell clusters improves β-cell survival due to improved molecular transport in the cell clusters, leading to better insulin secretion and glycemic regulation in diabetic mouse models. This promising result also raises a question: the advantage of encapsulating smaller cell clusters for enhanced molecular transport appears to contradict the use of larger microcapsules, which are less effective for molecular transport. Certainly, the long-term cell survival and cluster stability for clinical applications need further efforts.

2.1.2 | Microfluidic Systems

Microfluidic systems are widely used to produce droplets quickly on a large scale (Hou et al. 2017). Two phases, including an aqueous solution and an organic phase, are used to generate water-in-oil droplets (Figure 1b). Once the droplets are formed, the polymers in the aqueous phase can be induced to solidify with numerous mechanisms, such as photoinitiated polymerization and ionic crosslinking. By controlling fluid flow patterns and optimizing microchannel design, one can vary the size of droplets at the microscale level (Niculescu et al. 2021). The cells can be encapsulated through microfluidic systems when suspended in the aqueous phase, as exemplified in an early study (Koh et al. 2002). Building on this foundation, a modular drop-based microfluidic system that advanced encapsulation by isolating single cells in picoliter droplets was introduced (Köster et al. 2008). The system was fabricated through soft lithography and bonded via oxygen-plasma activation for precision and stability. While the system efficiently encapsulates single cells in picoliter droplets, it requires precise control of flow rates and nozzle dimensions, making it sensitive to operational variability.

While microfluidic systems were initially designed for single mammalian cell encapsulation, an advanced high-throughput microfluidic device for the mass production of alginate-microencapsulated islets was developed (Tendulkar et al. 2011). The high-throughput device addressed the challenges in scalability and islet viability for transplantation. The microfluidic device was fabricated to create a scalable system for producing alginate microcapsules and encapsulating pancreatic islets. The device features a concentric nozzle design where a sodium alginate-cell suspension flows through an inner nozzle and is sheared by compressed air in the outer nozzle. This device allows for the production of a large number of droplets that further solidify in a calcium chloride bath. The transition from manual, small-scale encapsulation methods to automated microfluidic systems represents a significant milestone in the historical development of microencapsulation. This device builds on earlier efforts to make encapsulation techniques more reliable and clinically viable, marking a critical step in the progression of the field.

This microfluidic system was further developed into a flow-focusing system for coating islets (Figure 1c; Tomei et al. 2014). The microcapsules were formed in thin, uniform layers (10–50 μm) via dithiothreitol-mediated cross-linking at acidic pH. While effective in preserving islet function and enabling transplantation, this direct method compromised viability and scalability due to the use of nonphysiological pH and viscosity

enhancers. Based on the development, another method involving an emulsion cross-linking method was developed. It uses a water-in-oil emulsion at neutral pH, pre-cross-linking PEG-maleimide with PEG-dithiol to achieve uniform coatings (18–28 μm) without additives (Stock et al. 2022). The emulsion method significantly improved cytocompatibility, maintained high islet viability, and increased scalability with a fivefold higher throughput than the direct method.

2.1.3 | Interfacial Polymerization

Islet microencapsulation can also be achieved through interfacial polymerization on the cell surface. Interfacial polymerization is a chemical process in which a polymer forms at the interface between two immiscible phases, typically involving the reaction of monomers dissolved in each phase at the interface (Morgan 2011). Unlike bulk polymerization, this technique offers remarkable versatility in tailoring the surface topology and chemical characteristics of the resulting functional materials. In addition, one noticeable advantage of this method is that it can avoid generating empty microcapsules compared to droplet-based encapsulation methods. The Hubbell group reported early demonstrations for coating islet surfaces using interfacial photopolymerization of PEG-based macromers (Cruise et al. 1999; Cruise, Hegre, Scharp, and Hubbell, 1998a; Cruise, Scharp, and Hubbell, 1998b; Sawhney et al. 1994). Islets were incubated in a solution of a photoinitiator such as eosin Y. As a trace amount of the photoinitiator was on the cell surface, mixing with a solution containing tetraacrylated PEG and triethanolamine would induce polymerization at the islet-solution interface once the cell suspension was exposed to and activated by a light source. The crosslinked macromers formed a hydrogel coating on the cell surface with a thickness of 10–50 μm. The viability of islets was ~86% after interfacial polymerization, indicating that free radicals and the procedure of interfacial polymerization did not cause significant damage to the encapsulated islets.

An alternative method was developed to encapsulate cells in thin, tunable microgels using interfacial polymerization (Figure 1d,e; Mao et al. 2017). Instead of using a photoinitiator displayed on the cell surface, calcium carbonate particles were deposited directly onto the cell surface as a localized calcium source. The cells run through a microfluidic device where acetic acid in the oil phase triggers calcium release from the nanoparticles. This leads to localized alginate crosslinking and the formation of a thin (~6 μm) hydrogel shell around each individual cell. This approach significantly improves encapsulation efficiency (over 90%) and allows precise control over hydrogel mechanical properties. However, this method has to rely on the usage of an organic solvent and acidic conditions. The reliance on acid to release Ca^{2+} in inducing alginate gelation may lead to reduced cell viability, particularly for islet cells that are highly sensitive to low pH.

2.2 | Materials

Recent advances in material science have enabled the development of novel biomaterials and their derivatives for cell encapsulation. These biomaterials, which include both natural polymers (e.g., alginate) and synthetic polymers (e.g., PEG), are used to address key challenges such as fibrotic overgrowth and immune cell activation. By tackling these issues, these materials aim to create an optimal microenvironment that supports the survival and function of transplanted islets.

2.2.1 | Alginate

Alginate is the most common polymer used for cell encapsulation. It is a natural polysaccharide primarily extracted from brown seaweed. It consists of linear copolymers of (1,4)-linked β-D-mannuronic acid (M) and α-L-guluronic acid (G) arranged in blocks (Figure 2a; Lee and Mooney 2012). The proportions of these blocks vary depending on the algal source, which influences its physical properties. Alginate is known for its biocompatibility, nontoxicity, and ability to form hydrogels in mild conditions through ionic cross-linking with divalent cations like calcium (Ca^{2+}). Gelation occurs when the G-blocks interact with calcium ions in an "egg-box" structure, forming a stable three-dimensional network. Factors such as G-to-M ratio, molecular weight, pH, and calcium concentration significantly affect the gelation process and the resultant gel's mechanical strength and stability. However, alginate gels can be dissolved under physiological conditions primarily due to ion exchange, where divalent ions in the gel are replaced by monovalent ions like sodium (Na^{+}).

Alginate contains impurities that can induce undesired immune responses. It requires purification or chemical modification for improving biocompatibility. Mounting evidence has shown that chemically modified alginate demonstrates superior performance with mitigated fibrosis and immune responses compared to unmodified alginate (Bochenek et al. 2018; Vegas, Veiseh, Doloff, et al. 2016). For example, alginate with zwitterionic modification induced less foreign body response and inhibited protein adsorptions due to enhanced antifouling (Li et al. 2022). Zwitterionic monomers such as sulfobetaine and carboxybetaine derivatives were applied to develop alginate microcapsules, demonstrating great potential in reducing fibroblast overgrowth and improving cell survival (Figure 2b; Liu et al. 2019). Another example of alginate modification is partial oxidation. This process involves treating ultrapure alginate with sodium periodate to introduce aldehyde groups, which facilitates hydrolysis and promotes degradation in vivo. Partially oxidized alginate shows advantages over unmodified alginate for islet encapsulation (Volpatti et al. 2023).

In addition to chemical modification, potent molecules can be physically encapsulated within the alginate hydrogel to regulate the immune response. CXCL12, a chemokine with immunoregulatory and anti-inflammatory properties, is known to reduce immune cell infiltration and mitigate the pericapsular fibrotic response (Alagpulinsa et al. 2019; Chen et al. 2015). Co-encapsulation of CXCL12 and cells in alginate microcapsules led to improved cell survival and enhanced insulin secretion functionality, showing the translational potential of improving cell microencapsulation and transplantation with local delivery of immunoregulatory molecules.

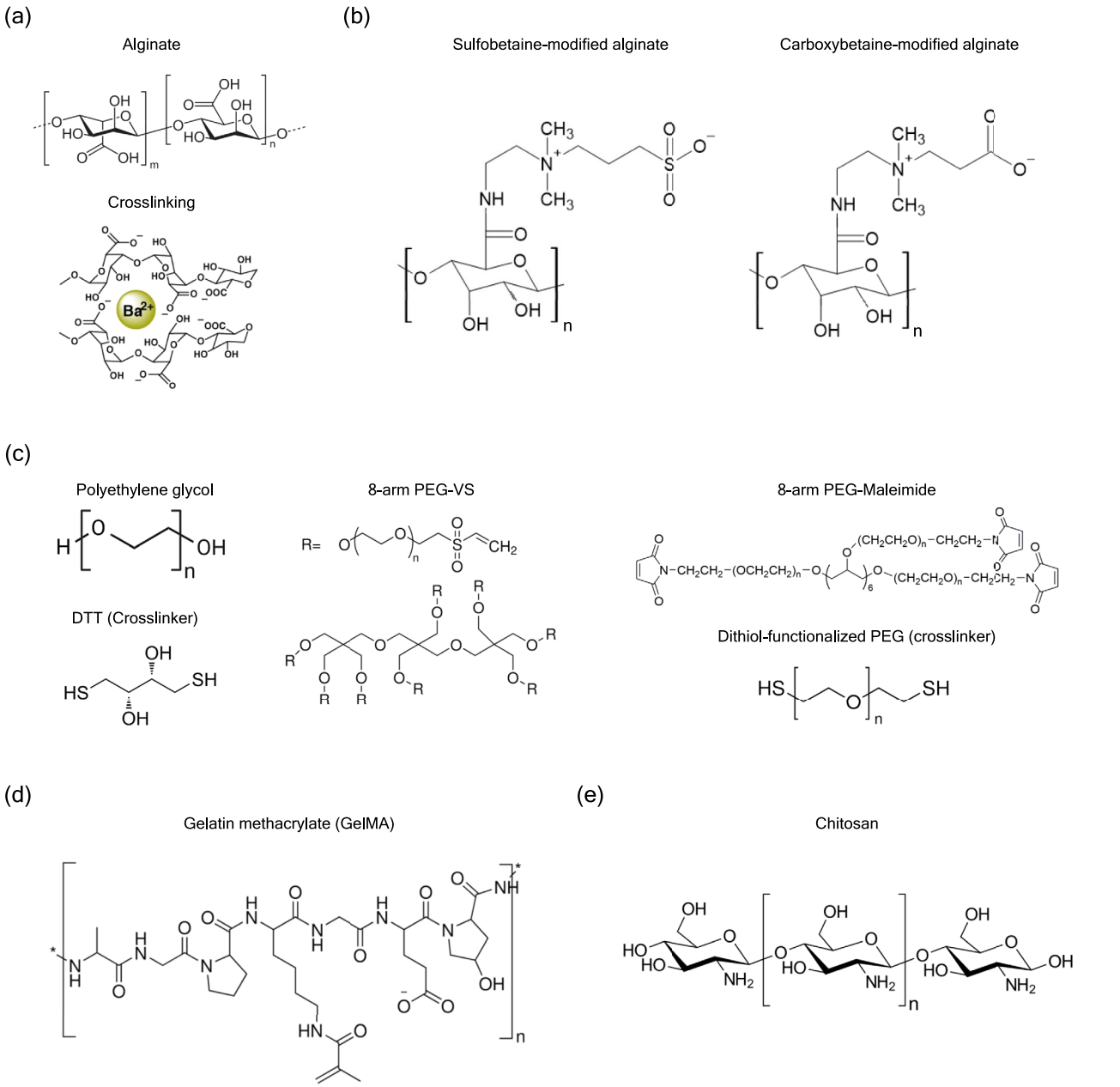

FIGURE 2 | Representative materials for islet microencapsulation. (a) Chemical structure of alginic acid and alginate crosslinking by bivalent cations such as Ca^{2+} or Ba^{2+}. Adapted from Liu et al. (2019). (b) Chemical structures of zwitterionically modified alginate. Sulfobetaine and carboxybetaine monomers were used for the conjugation. Adapted from Liu et al. (2019). (c) Chemical structures of PEG and PEG derivatives with functional groups. Multi-arm PEG derivatives can be crosslinked to form hydrogels via different addition reactions (Tomei et al. 2014; Stock et al. 2020). (d) Chemical structure of gelatin methacryloyl (GelMA) showing only representative amino acid residues in the backbone. (e) Chemical structure of chitosan, primarily composed of D-glucosamine units that are the deacetylated form of N-acetyl-D-glucosamine.

2.2.2 | Polyethylene Glycol (PEG)

PEG is a synthetic polymer composed of repeating ethylene glycol units, widely used for hydrogel formation due to its high solubility in water, molecular flexibility, thermal stability, and antifouling properties. PEG hydrogel has been used for cell microencapsulation because of its high solubility in water, molecular flexibility, thermal stability, and anti-fouling characteristics (D'souza and Shegokar 2016). The gelation of PEG can be controlled by various factors, including pH, temperature, and molecular weight, allowing for tunable mechanical properties and biocompatibility.

PEG hydrogels can be formed through multiple polymerization techniques. One commonly used method is interfacial photopolymerization, where PEG macromers are crosslinked upon exposure to light in the presence of a photoinitiator (D'souza and Shegokar 2016). This approach enables the formation of a conformal hydrogel layer around islets, ensuring minimal diffusion barriers while maintaining the structural integrity of the encapsulated cells. Beyond photopolymerization, Michael addition chemistry provides an alternative strategy for PEG hydrogel synthesis (Tomei et al. 2014). Multi-arm PEG derivatives, such as PEG-vinyl sulfone (PEG-VS), can undergo crosslinking with thiol-containing crosslinkers like dithiothreitol

(DTT; Figure 2c). Similarly, maleimide-functionalized PEG and dithiol-functionalized PEG (SH-PEG-SH) can be crosslinked via Michael addition, forming a stable hydrogel network (Stock et al. 2020, 2022).

The kinetics of PEG hydrogel formation is highly dependent on pH, particularly in thiol-based Michael addition reactions (D'souza and Shegokar 2016). In a basic environment, deprotonation of thiol groups enhances their nucleophilicity, accelerating crosslinking and hydrogel formation. However, while fast gelation is essential for maintaining cell viability, excessive exposure to a basic pH environment may compromise cell integrity. This challenge underscores the need for optimized reaction conditions that balance rapid hydrogel formation with cytocompatibility. Additionally, rapid separation of encapsulated cells from the reaction mixture is critical to preventing prolonged exposure to potentially cytotoxic conditions.

2.2.3 | Extracellular Matrix (ECM)

When the islets of Langerhans are separated from the pancreas, enzymes such as collagenase and neutral proteases are used to digest the surrounding exocrine tissue. However, using these enzymes inevitably causes the damage to the ECM surrounding the islets. The ECM plays a critical role in maintaining the structure and function of pancreatic islets (Theocharis et al. 2016). As a physical scaffold, the ECM preserves the three-dimensional architecture of the islets, ensuring proper cell-to-cell communication and coordinated insulin secretion (Llacua et al. 2018). The ECM also contributes to the mechanical stability of the islets, and their loss can lead to fragmentation and structural alterations that compromise islet integrity. Beyond structural support, the ECM regulates cellular functions by interacting with surface receptors of islet cells and influences intracellular signaling pathways that regulate insulin secretion. ECM disruption has been found to reduce the responsiveness of islets to glucose, leading to decreased insulin secretion. As the ECM is fundamental not only to the structural integrity of islets but also to their survival, function, and successful integration, studies have been conducted to use the ECM or ECM components for islet encapsulation and transplantation (Theocharis et al. 2016).

Decellularization is commonly used to acquire the pancreatic ECM (Sackett et al. 2018). This process involves hypertonic and hypotonic treatments, followed by lyophilization and pepsin digestion to create a bioactive hydrogel. The decellularized ECM can be further combined with alginate to be crosslinked with calcium chloride to encapsulate islets in a biomimetic pancreatic microenvironment. This ECM-based microencapsulation enhanced islet viability, glucose responsiveness, and graft performance while mitigating cytokine-induced cytotoxicity compared to conventional alginate microcapsules.

In addition to using the whole ECM, effort has also been made to use ECM components or derivatives for cell encapsulation. Gelatin, a major structural protein in the ECM, is derived from collagen (Alipal et al. 2021). Gelatin has excellent biocompatibility and mechanical tunability. Its hydrophilic nature supports cell viability and proliferation by providing a nutrient-permeable environment. Gelatin can be chemically modified with methacrylate to acquire a conjugate, that is, GelMA, that can undergo photo-crosslinking by itself or with monomers to form hydrogel (Figure 2d; Sun et al. 2023). Porous zwitterionic GelMA microgels have been studied via a microfluidic approach for islet encapsulation and transplantation (Xiao et al. 2022).

2.2.4 | Others

The process of islet encapsulation often involves multiple polymers to enhance the mechanical integrity, biocompatibility, and/or immunoisolation properties of the microcapsules. In addition to alginate, PEG and ECM, other polymers were used for microencapsulation (Desai and Shea 2017; Wang et al. 2024). For instance, when alginate is used as the primary encapsulating material, positively charged polymers such as poly-L-lysine (PLL), chitosan, and poly-L-ornithine (PLO) are commonly applied to form polyelectrolyte complexes with the negatively charged alginate (Figure 2e). Notably, PLL and PLO are synthetic polymers, while chitosan is a natural cationic polysaccharide derived from chitin with excellent biocompatibility and biodegradability (Hu et al. 2021).

2.3 | In Vivo Studies

In the pioneering study by Lim and Sun, encapsulated islet recipients maintained normoglycemia for up to 3 weeks, while unencapsulated islets failed within 1 week (Lim and Sun 1980). This result inspired extensive research into refining microcapsule synthesis. Improvements such as using low-viscosity alginate for more uniform droplet formation and increasing the alginate-polylysine membrane thickness enhanced mechanical strength, extending islet survival in diabetic models to nearly 1 year (O'Shea et al. 1984). The use of zwitterionic alginate was also found to improve the quality of microcapsules in reduced fibrosis and foreign body responses (Liu et al. 2019; Vegas, Veiseh, Gürtler, et al. 2016). Diabetic mice receiving encapsulated human stem cell-derived β-cells maintained normoglycemia for over 174 days without systemic immunosuppression (Vegas, Veiseh, Gürtler, et al. 2016). To demonstrate the potential of alginate-based microcapsules, xenogeneic adult porcine islets encapsulated in alginate microcapsules were transplanted into diabetic nonhuman primates (NHPs). The graft remained functional for up to 70 days, with detectable C-peptide for up to 125 days (Figure 3a; Safley et al. 2018). Encapsulated porcine islets improved fasting glucose levels and reduced hemoglobin A1c (HbA1c), confirming their viability and functionality in diabetic NHP models. In addition, a team in Brussels could achieve 6-month insulin independence in nonhuman primates using both micro- and macro-encapsulation approaches (Dufrane et al. 2006, 2010). Encouraging results observed in large animal studies were also seen in early clinical trials. For example, when neonatal porcine islets encapsulated in alginate-poly-L-ornithine–alginate (i.e., DIABECELL) were transplanted into patients with T1D and two doses were administered 3 months apart, patients maintained HbA1c levels below 7% for over 600 days, with significantly fewer hypoglycemic events and no virus transmission or severe adverse effects (Figure 3b;

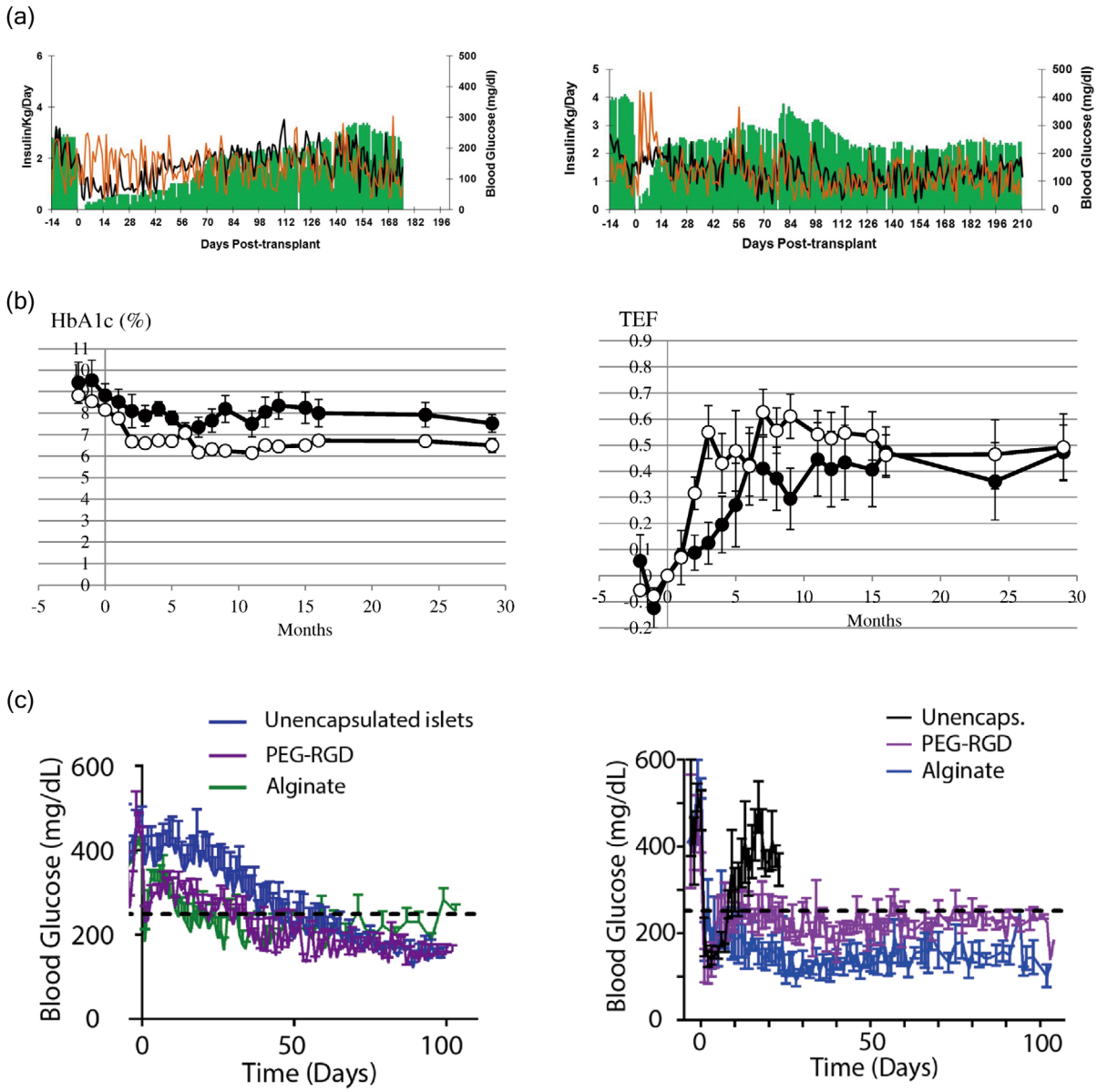

FIGURE 3 | In vivo evaluation of microencapsulated islets. (a) Blood glucose monitoring of diabetic nonhuman primates receiving porcine islets. *Left*: Recipient ID# NHP 8C4-40, *Right*: Recipient ID# NHP 8C4-16. Adapted from Safley et al. (2018). (b) Time progression profiles of HbA1c and TEF. Different groups are shown in (●) or (○). Encapsulated neonatal porcine islets were transplanted twice into type 1 diabetic patients. The second transplantation was conducted 3 months after the initial procedure. Adapted from Matsumoto et al. (2016). (c) Blood glucose monitoring of diabetic mice after syngeneic (left) and allogeneic (right) islet transplantation. Adapted from Weaver et al. (2019).

Matsumoto et al. 2016). This technology is still under development by Otsuka Pharmaceutical Factory Inc. Up to date, most clinical islet xenotransplantations have been conducted using encapsulation to avoid the need for systemic immunosuppression (Piemonti et al. 2024).

PEG-based microencapsulation has also been explored as an alternative. PEG was initially proposed for its nonimmunogenic properties and biocompatibility, with early studies showing that PEG coatings could extend graft survival by minimizing fibrosis and immune rejection (D'souza and Shegokar 2016). The therapeutic outcomes in animals transplanted with PEG-microencapsulated islets were comparable to those with alginate-based microencapsulation (Figure 3c; Weaver et al. 2019). Allogeneic diabetic animals receiving islets microencapsulated with either of the two biomaterials maintained normoglycemia for over 100 days, whereas unencapsulated islets were rapidly rejected within an average of 10–12 days. Even after a much thinner PEG hydrogel was applied to encapsulate xenogeneic porcine islets, the recipient rodents could restore normoglycemia without immunosuppression for over 100 days (Hill et al. 1997). PEG-based cell encapsulation was also studied in NHP studies. Unfortunately, the large animals were found to react aggressively to the PEG material, similar to the reaction to xenograft tissues (Scharp 2007). This immune recognition may be one of the reasons for losing the function of PEG-encapsulated islets. Indeed, while PEG was initially believed to be nonimmunogenic, emerging studies revealed that PEG can elicit immune responses, leading to anti-PEG antibody production and

complement activation (Chen et al. 2021; Ibrahim et al. 2022). To address these issues, low-dose cyclosporine was administered to diabetic baboons for 30 days. PEG-encapsulated islets reversed hyperglycemia for 14–20 months in 3 out of 5 recipients (Scharp et al. 2005). The first Phase I/II clinical trial (NCT00260234) for PEG-encapsulated islets was conducted by Novocell (2005). Unfortunately, the trial was terminated due to limited efficacy observed in the initial participants.

3 | Nanoencapsulation

As molecular transport in large microcapsules is a concern for long-term cell survival, efforts have also been made in reducing the size or volume of polymers used to shield islets. Nanoencapsulation leverages nanoscale coating techniques to overcome the challenge of limited molecular transport. While many nanoencapsulation methods have similarities to microencapsulation, they have their intrinsic characteristics in formation and function.

3.1 | Methods

3.1.1 | Layer-By-Layer Coating

The most common method for cell nanoencapsulation is layer-by-layer (LbL) coating, which constructs thin films through the sequential adsorption of complementary molecules, such as polyelectrolytes, proteins, nanoparticles, and biomacromolecules (Hammond 2012). This molecular adsorption is driven by electrostatic interactions, hydrogen bonding, hydrophobic forces, or covalent bonding, enabling precise regulation of nanometer-scale thickness (Figure 4a). A key advantage of the LbL method is its water-based, room-temperature assembly process, which eliminates the need for complex instrumentation or harsh conditions. This gentle approach not only preserves the viability of coated living cells but also ensures 100% coating efficiency, preventing the formation of empty capsules or uncoated cells.

Electrostatic interaction-based LbL nanoencapsulation is the most widely used approach, where alternating layers of oppositely charged molecules are deposited around islets to form a stable nanocoating (Krol et al. 2006). This method allows for precise control over capsule permeability, enabling immune isolation while maintaining efficient nutrient and insulin exchange. By modifying the number of deposited layers and the ionic strength of the solutions, the encapsulation can be tailored to optimize protection and functionality. Additionally, electrostatic LbL assembly can integrate multiple cell types, enabling the formation of pseudoislets with enhanced structural integrity and immune resistance (Figure 4b; Bhaiji et al. 2012; Zhi et al. 2012). However, a key challenge of this method is the potential instability of the nanocoating in physiological conditions, as the layers may degrade over time, compromising their protective function.

Covalent LbL assembly via enzymatic cross-linking offers an alternative to electrostatic deposition by providing greater structural stability. This method involves the sequential dipping of islets or cell spheroids into precursor solutions, followed by an enzyme-catalyzed cross-linking reaction that covalently bonds the layers together. For example, glycol chitosan and hyaluronic acid were conjugated with monophenol residues for cross-linking in the presence of *Streptomyces avermitilis* tyrosinase (Kim et al. 2021). This enzyme-aided residue oxidation and functional cross-linking allow for durable hydrogel nanofilm formation, which enhances coating stability without compromising high permeability for nutrient transport and insulin secretion. Covalent LbL coating can also be achieved without using enzymes. For example, dendritic polymer-based LbL nanocoating utilizes silane self-assembly and covalent stabilization through functionalized dendrimers, forming a highly controlled nanoshell around islets (Gattás-Asfura et al. 2020). The covalent bonding between dendritic layers prevents premature degradation, making it a promising strategy for long-term encapsulation stability. However, this method presents challenges such as potential cytotoxicity and cellular disruption, which may limit its clinical translation.

3.1.2 | One-Step Assembly and Crosslinking

The LbL method relies on the iterative dipping of cells into alternating solutions to gradually build nanocoatings. While this technique allows for precise control over coating composition and structure, it presents several challenges. First, achieving a desired coating thickness requires multiple deposition cycles, which can compromise cell viability due to prolonged exposure to external conditions and mechanical stress from repetitive handling. Additionally, studies have shown that despite the iterative molecular adsorption process, each deposition cycle typically results in only a few nanometers of thickness per layer. As a result, even after numerous cycles, the final coating may not be sufficiently thick to provide adequate mechanical protection or immunoisolation. These limitations highlight the need for alternative deposition strategies that balance coating stability, functional performance, and cell viability.

A recently developed biomimetic cell wall (BCW) offers a promising alternative to LbL encapsulation, providing a structurally robust yet biologically adaptive approach to cell coating (Shi et al. 2019). This method employs supramolecular DNA assembly and alginate–polylysine complexation to generate a stable nanocoating (70–150 nm thick) around mammalian cells (Figure 4c). Notably, both DNA assembly and complexation occur in a single-step process, streamlining the encapsulation procedure. The method is initiated by inserting cholesterol-conjugated DNA initiators into the cell membrane, which trigger a hybridization chain reaction to form an extended DNA-alginate scaffold around the cell. This scaffold subsequently serves as a template for crosslinking alginate-DNA conjugates with polylysine via polyelectrolyte complexation, completing the encapsulation in a highly efficient manner. The resulting BCW structure enhances cell protection against physical and biological assaults while preserving molecular transport essential for cell function. Beyond structural reinforcement, the BCW can be further functionalized through the physical immobilization of nanoparticles, offering a versatile platform for cell-based therapies and advanced bioengineering applications (Lee et al. 2023). This technique has been explored for in vivo mesenchymal stem

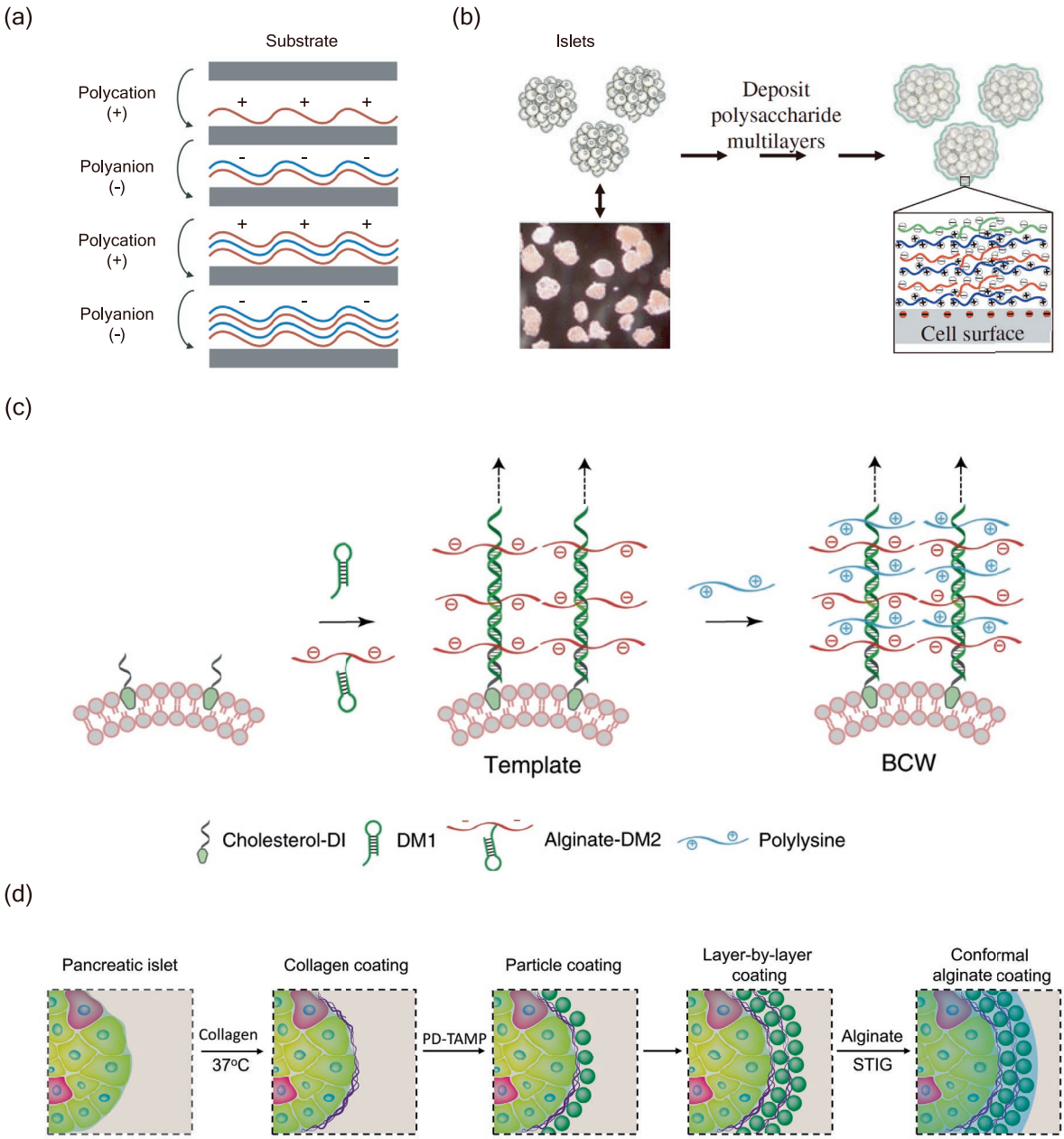

FIGURE 4 | Methods for cell nanoencapsulation. (a) The iterative layer-by-layer coating process involves the sequential deposition of oppositely charged biomaterials onto the islet surface, forming stable multilayers. This technique enhances biocompatibility and protection by allowing precise control over layer composition and thickness. (b) The schematic illustrates nano-scale encapsulation of pancreatic islets through layer-by-layer deposition of charged polysaccharide multilayers. Chitosan-PC (+) is shown in blue, alginate (–) in red, and chondroitin-4-sulfate-PC (–) in green. Adapted from Zhi et al. (2012). (c) DNA-templated nanoencapsulation without iterative cell treatment. Cholesterol-conjugated DNA initiators are displayed on the cell surface, where they trigger the automatic assembly of DNA-polymer complexes, followed by polyelectrolyte complexation. Adapted from Shi et al. (2019). (d) The schematic illustrates a stepwise method for coating islets with collagen, PD-TAMP, and alginate. Islets were first incubated in collagen solution at 37°C to facilitate collagen fiber formation and PD-TAMP binding, followed by sequential layering of PD-TAMP and collagen. Lastly, a thin alginate hydrogel layer was applied via surface-triggered in situ gelation (STIG) to stabilize the coating. Adapted from Le Tran et al. (2022).

cell transplantation, demonstrating enhanced cell survival compared to uncoated cells. However, despite the initial benefits, nanoencapsulated cells exhibited a 70% loss in activity within 1 week. Additional refinements of BCW-based nanoencapsulation are required for long-term applications, particularly in islet transplantation.

3.1.3 | Direct Conjugation

The cell surface is a substrate enriched with primary amine (–NH_2), thiol (–SH, cysteine), carboxyl (–COOH), and hydroxyl (–OH) functional groups, which play essential roles in cell interactions and signaling. They can also be used to react with

exogenous molecules or nanoparticles for cell nanocoating via amine-carboxyl, thiol-maleimide, or click chemistry reactions.

For instance, N-hydroxy succinimide-functionalized PEG valeric acid (SVA-PEG) was used to modify the surface of islets for PEGylation (Nguyen et al. 2021). By incubating islets in SVA-PEG solution at pH 8.0 for 1 h, stable covalent linkages with cell surface amines were formed, effectively masking surface antigens to reduce immune recognition. In addition to molecules, nanoparticles can be directly conjugated to the cell surface for coating (Kim et al. 2020; Le Tran et al. 2022). Polydopamine-coated poly(lactic-co-glycolic acid) microparticles with tacrolimus loading (PD-TAMP) have also been explored for islet surface modification. PD-TAMPs are synthesized through solvent displacement, where PLGA forms the hydrophobic core, PEG provides biocompatibility, and polydopamine enables surface adhesion. During islet incubation in an alkaline solution (pH 8.0), catechol groups in polydopamine undergo oxidation to quinones, allowing covalent bond formation with amine groups in the islet's collagen, effectively modifying the surface properties (Figure 4d).

These approaches highlight the potential of chemical conjugation strategies for islet surface modification, offering opportunities to enhance cell survival, immune evasion, and functional integration in transplantation applications. However, further optimization of reaction conditions, nanoparticle formulations, and biocompatibility assessments is crucial for translating these technologies into clinical practice. In particular, ensuring cell viability under nonphysiological reaction conditions remains a key challenge that must be addressed to maintain islet functionality and therapeutic efficacy.

3.2 | Materials

Materials traditionally used for islet microencapsulation have also been adapted for nanoencapsulation, but with distinct modifications to meet the unique demands of nanoscale applications. One key difference lies in the chemical modifications that enable enhanced functionality at the nanoscale. For instance, alginate is commonly modified in microencapsulation to reduce fibrosis and immune responses. In nanoencapsulation, alginate can be conjugated with DNA to facilitate self-assembly processes (Shi et al. 2019). This DNA-conjugated alginate leverages the hybridization chain reaction to form supramolecular frameworks, offering precise control over the encapsulation structure and improved protection.

Another negatively charged biopolymer used for nanoencapsulation is heparin which brings unique biochemical properties to the encapsulation process (Hong et al. 2013). Heparin exhibits strong electrostatic interactions with cationic polymers for LbL assembly (Park et al. 2018). Its pentosan backbone, enriched with carboxyl, sulfonic acid, and hydroxyl groups, contributes to its high solubility and binding affinity for various biomolecules. Beyond its structural properties, heparin's anticoagulant activity and biocompatibility are, in principle, beneficial to eliminate the undesired immune response. For example, when heparin was chemically conjugated with other polymers to form a thin, biocompatible nanocapsule on the islet surface, it effectively mitigated instant blood-mediated inflammatory reactions and improved graft survival (Lou et al. 2017).

Cationic and anionic ECM proteins such as fibronectin and gelatin have been utilized to form nanocoatings on insulin-secreting cells using LbL assembly techniques (Fukuda et al. 2018). These materials are highly biocompatible, exhibit low cytotoxicity, and significantly enhance cell adhesion and intercellular interactions. Collagen can self-assemble into uniform layers on mammalian cell surfaces (Le Tran et al. 2022). The properties of collagen coatings can be fine-tuned by crosslinking with bioactive molecules or synthetic polymers, allowing for precise control over mechanical strength and degradation rates to optimize cell function and long-term encapsulation stability. However, as nanocoatings are thin and these natural proteins are prone to rapid enzymatic degradation in vivo, their ability to maintain long-term stability is a concern. This susceptibility may limit their effectiveness as a stable protective barrier for long-term islet survival.

PEG, as a synthetic polymer, was also studied for both micro- and nanoencapsulation, but with significant differences in its form and function. Conventional PEG hydrogels used in microencapsulation form bulk crosslinked matrices around islets (D'souza and Shegokar 2016). In contrast, lipid-conjugated PEG was utilized in nanoencapsulation. With the phospholipid moiety, lipid-conjugated PEG spontaneously anchors to the islet surface through hydrophobic interactions with the lipid bilayer (Teramura and Iwata 2009). This assembly results in a thin coating on the surface of the islets.

PEG was also conjugated with other polymers to improve biocompatibility. PLL is known to induce plasma membrane pore formation and subsequent cell death (Hong et al. 2006). Conjugation of PEG to PLL significantly reduces this membrane-disruptive property. This modification not only decreases cytotoxicity but also prevents the unintended transport of molecules across the cell membrane. Additionally, PEG groups can be functionalized with thiol (–SH), amine ($–NH_2$), or carboxyl (–COOH) groups, facilitating site-specific conjugation and crosslinking. Thiol-functionalized PEG (PEG-SH) promotes disulfide bond formation and Michael addition reactions, while multi-arm derivatives such as SH-6-arm-PEG-NHS provide multiple reactive sites, enhancing crosslinking efficiency and stability in nanoencapsulation systems (Haque et al. 2017).

3.3 | In Vivo Studies

Islet nanoencapsulation has been studied for many years, but overall therapeutic outcomes have not proven to be as promising as those achieved with microencapsulation. In most animal studies, animals could maintain normoglycemia for approximately 1 month. A few examples of nanoencapsulation are discussed as follows.

Dendrimer-based nanocoating via the covalent LbL technique was applied to islet nanoencapsulation and transplantation (Gattás-Asfura et al. 2020). The nanoencapsulated islets restored

normoglycemia in NOD-SCID immunodeficient mice for over 90 days while maintaining high metabolic activity and insulin secretion, comparable to nonencapsulated islets. These results demonstrate that the nanoencapsulation process, as well as the dendrimer used, did not compromise cell function or viability. However, further studies in immunocompetent animal models are needed to fully evaluate the long-term efficacy and immune protection offered by this promising nanoencapsulation system. Similarly, nanoencapsulation of islets using phosphorylcholine-modified chitosan, alginate, and chondroitin-4-sulfate was also evaluated in vivo (Zhi et al. 2012). Animals transplanted with nanoencapsulated islets maintained normoglycemia for 28–37 days in allogeneic models, a significantly improved outcome compared to nonencapsulated controls (Figure 5a). Notably, two out of seven mice experienced early graft rejection, possibly due to incomplete or degraded coatings. Recently, nanoencapsulated pancreatic β-cell spheroids were transplanted into immunocompetent diabetic mice (Kim et al. 2021). The results showed that the recipients with nanoencapsulated cells exhibited a reduction in blood glucose levels from hyperglycemia to normoglycemia (Figure 5b). Nephrectomy of the kidney containing the nanoencapsulated spheroids caused an immediate return to hyperglycemia, confirming that glycemic control was mediated by the transplanted β cells. However, this therapeutic effect was only maintained for up to 30 days. Nanoencapsulation of human islets using the chitosan and polystyrene sulfonate layer-by-layer method was also evaluated in vivo (Syed et al. 2018). Similarly, the recipients restored normoglycemia for up to only 1 month without the need for immunosuppressive drugs (Figure 5c).

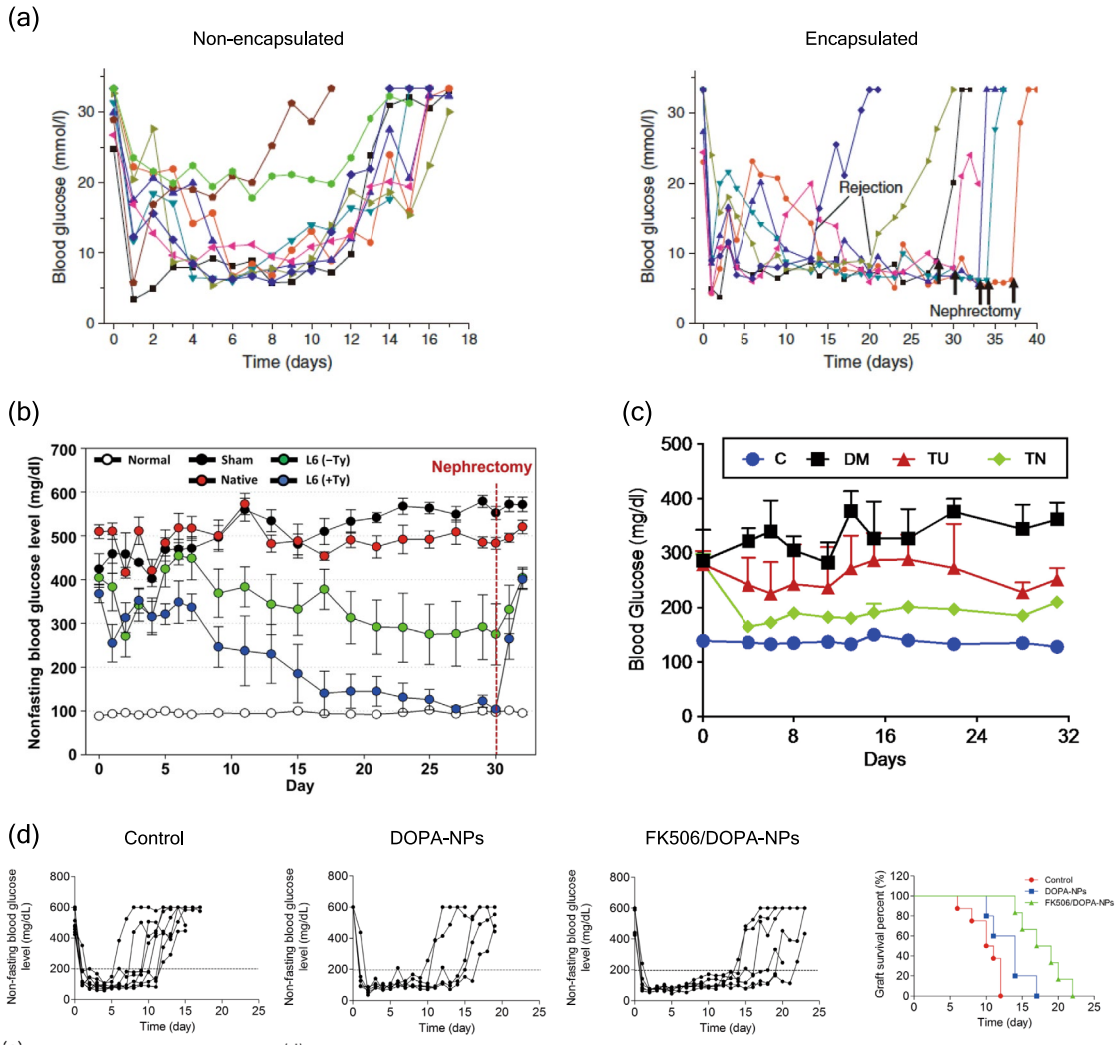

FIGURE 5 | Therapeutic outcomes of nanoencapsulated islets. (a) Allogeneic transplantation of both nonencapsulated and nanoencapsulated Balb/c islets successfully reversed STZ-induced hyperglycemia in diabetic C57BL/6 mice. In the right graph, upright arrows indicate the day of nephrectomy. Adapted from Zhi et al. (2012). (b) Non-fasting blood glucose levels in β cell spheroid transplanted mice. Histological examination of implants collected 30 days post-implantation from streptozotocin-treated BALB/C mice, shown through hematoxylin and eosin (H&E) staining and insulin immunostaining in each group. Scale bars: 200 μm (top images) and 100 μm (bottom images). Adapted from Kim et al. (2021). (c) Nonfasting blood glucose levels in control mice (C), untreated diabetic mice (DM), diabetic mice transplanted with uncoated human islets (TU), and diabetic mice receiving nanoencapsulated human islets (TN). Adapted from Syed et al. (2018). (d) Nonfasting blood glucose levels in diabetic C57BL/6 mice following transplantation of control islets, DOPA-NPs-coated islets, or FK506/DOPA-NPs-coated islets. Graft survival rates for each group are shown. Adapted from Pham et al. (2018).

The short-term diabetes treatment was likely due to the strong immune response in the transplantation site and the inability to use nanocoating to protect encapsulated cells. Thus, to suppress the local immune system and improve cell survival, studies were carried out to coat the islet surface with immunosuppressant drug-loaded 3,4-dihydroxyphenethylamine conjugated PLGA-PEG nanoparticles (DOPA-NPs). Modified rat islets with FK506-loaded polymeric nanoparticles were transplanted in an immunocompetent diabetic C57BL/6J mice model (Pham et al. 2018). Unfortunately, the modified islets restored normoglycemia in diabetic mice for only a median graft survival time of 18 days, compared to 10.5 days for uncoated islets and 15 days for islets coated only with nanoparticles (Figure 5d).

4 | Hypoimmune Engineering

Transplanted islets are recognized as foreign cells by the immune system of a recipient, which initiates an immune response for rejection. Strategies for protecting islets have been studied to address this challenge using immunosuppressants, biomaterials, and devices. Researchers also used genetic engineering tools to modify cell surfaces to evade detection and destruction by the immune system. Alternatively, researchers developed a biomimetic immune-tolerant microenvironment that can support the survival and function of transplanted islets. These methods are called hypoimmune engineering.

4.1 | Key Technologies

4.1.1 | Removal of Surface Antigens

A key strategy employed in recent years involves the direct modification of transplanted islets to reduce immunogenicity by removing key antigen-presenting molecules expressed on the cell surface. Among these, human leukocyte antigen (HLA) Class I molecules—comprising HLA-A, HLA-B, and HLA-C—are critical targets due to their role in presenting antigens to CD8+ T cells, thereby eliciting a cytotoxic immune response (Figure 6a) (Parent et al. 2021). Similarly, HLA Class II molecules—consisting of HLA-DP, HLA-DQ, and HLA-DR—are targeted for their ability to present antigens that activate CD4+ T cells and amplify the immune response.

The deletion of HLA Class I and Class II molecules in human pluripotent stem cells (hPSCs) has been studied for potential diabetes treatment due to their ability to differentiate into beta cells (Parent et al. 2021). However, the deletion of HLA Class I molecules can trigger natural killer (NK) cell activation, leading to graft rejection. To address this issue, HLA-A2 expression was selectively preserved while the remaining HLA Class I and Class II molecules were eliminated. In this approach, the cells underwent multiple cycles of electroporation with Cas9/gRNA ribonucleoproteins (RNPs), a delivery method that uses electrical pulses to introduce specific genes or proteins into cells (Cheng et al. 2022). The CRISPR–Cas9 system enables precise gene knockout or targeted modifications by introducing double-stranded breaks in DNA (Mengstie and Wondimu 2021; Ran et al. 2013). In the first cycle of modification, the expression of all HLA Class I and Class II molecules was reduced, allowing for the sorting of cells with normal HLA-A2 expression. A subsequent cycle of modification specifically targeted HLA-B, HLA-C, and HLA Class II molecules for deletion. A degranulation assay demonstrated that the engineered cell line expressing HLA-A2 suppressed NK cell activation, confirming the efficacy of retaining HLA-A2 expression to prevent immune responses when knocking out other HLA Class I molecules (Parent et al. 2021). While the effects of multiple electroporation cycles on gene delivery efficiency, cell transfection success rates, and long-term cellular bioactivity remain elusive, antigen removal methods have shown significant potential in reducing immune attacks.

4.1.2 | Display of Protective Molecules

The insertion of self-protective molecules on the surface has been proven to be a powerful strategy to regulate T-cell responses and promote graft tolerance (Arakaki et al. 2014; Yu et al. 2016). Key self-protective molecules include FasL and PD-L1, each of which plays a distinct role in immune modulation. FasL induces activation-induced cell death (AICD), a natural process that eliminates overactivated T cells to prevent excessive immune responses and maintain immune homeostasis (Figure 6b; Yolcu et al. 2011). This is particularly critical in transplantation, where the recipient's immune system is often hyperactive in response to foreign antigens. PD-L1, on the other hand, is a pivotal immune checkpoint molecule that inhibits T-cell activation by engaging the PD-1 receptor on T cells (Figure 6b; Batra et al. 2020). This interaction not only suppresses effector T-cell activity but also promotes the development of regulatory T cells (Tregs), which are essential for maintaining long-term immune tolerance.

Studies have utilized these mechanisms to engineer transplanted islets with self-protective molecules. Yolcu et al. developed a streptavidin-FasL chimera (i.e., SA-FasL) to induce T-cell apoptosis and enhance immune tolerance (Yolcu et al. 2011). Their strategy utilized streptavidin-based molecular engineering, where the extracellular domain of FasL was fused to streptavidin (SA) to produce a chimeric fusion protein. To display this molecule on the cell surface, islets undergo biotinylation, that is, chemical conjugation of biotin to the surface of islets. The chemically conjugated biotin molecules on the cell surface allowed SA-FasL to be transiently displayed on biotinylated islets, effectively promoting immune regulation by inducing activation-induced cell death (AICD) in T cells. Batra et al. expanded this platform by employing streptavidin-based engineering to develop SA-PDL1, a chimeric protein designed to inhibit T-cell activation via engagement with the PD-1 immune checkpoint pathway (Batra et al. 2020). The transient display of PD-L1 on biotinylated islets provided an additional mechanism for promoting graft tolerance, addressing adaptive immune responses. While this approach significantly improved graft survival in animal models, it required chemical modification of the islet surface for biotinylation. The half-life of biotin molecules on the cell surface is short. Additionally, SA is of bacterial origin, and its long-term effects in vivo have yet to be fully evaluated.

To improve the display of the self-protective molecules, Yoshihara et al. expanded on this approach by integrating the

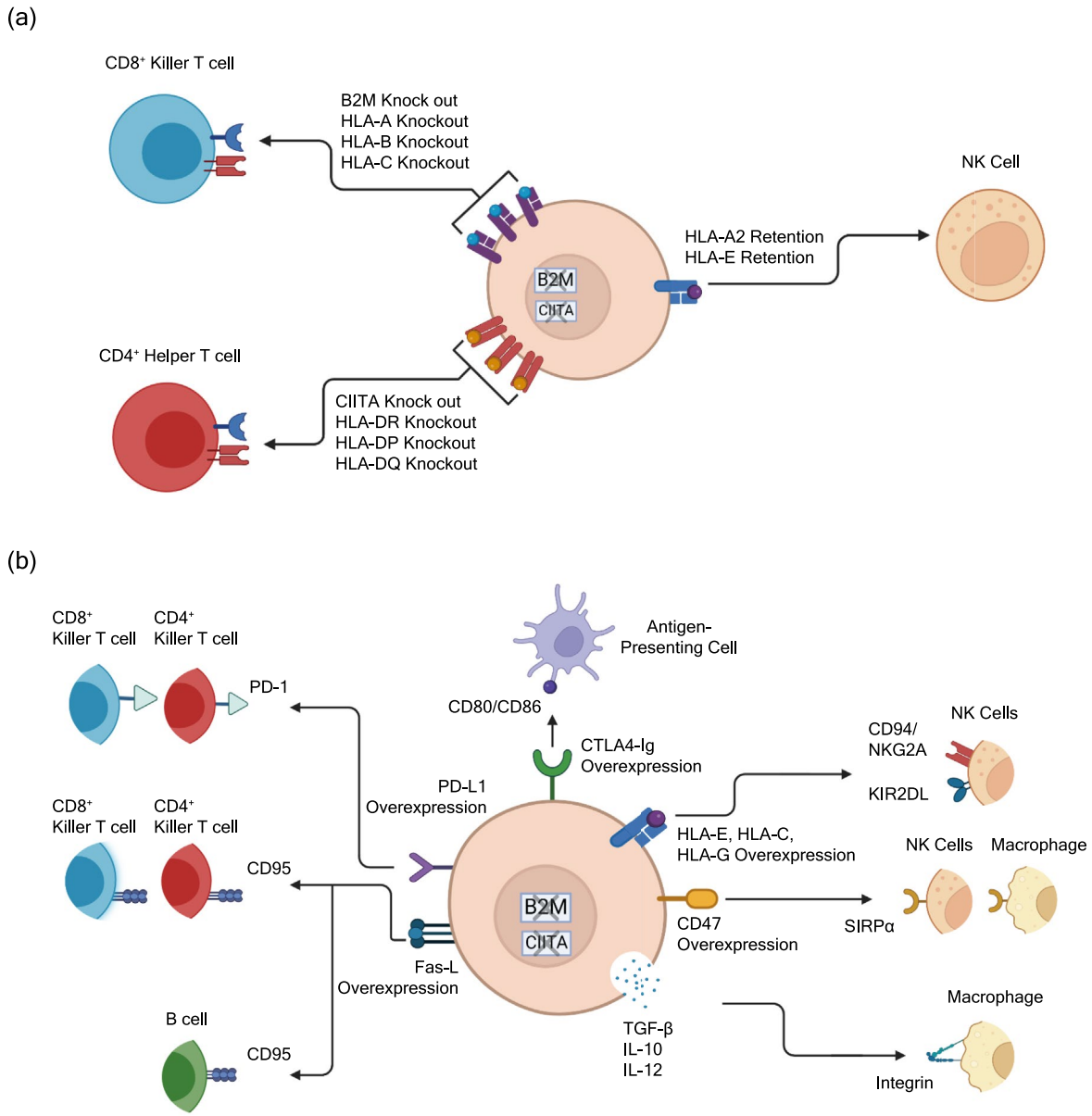

FIGURE 6 | Surface engineering via the Removal and/or display of specific molecules. (a) Selective removal and retention of cell surface proteins. The knockout of HLA class I and class II molecules enables immune evasion from $CD8^+$ cytotoxic T cells and $CD4^+$ helper T cells. Selective retention of specific HLA antigens prevents the activation of natural killer (NK) cells. (b) Expression of specific cell-protecting ligands or Secretory cytokines. Self-protective molecules, including PD-L1, FasL, CTLA4-Ig, HLA-E, HLA-C, HLA-G, and CD47, can be overexpressed on the cell surface, or secreted cytokines such as CD47, TGF-β, IL-10, and IL-12 can be released from cells to reduce immunogenicity.

expression of self-protective molecules through genetic modifications in human islet-like organoids (HILOs) derived from iPSCs (Yoshihara et al. 2020). A PD-L1-expressing lentiviral vector was used to confer immune protection, employing puromycin selection to enrich successfully transduced cells. Lentiviral vectors are an engineered delivery system derived from HIV-1 designed to integrate genetic material into host cells (Dull et al. 1998). These vectors are widely used due to their ability to deliver genes with high efficiency and support long-term expression, and they have been refined over successive generations to improve safety and minimize risks such as replication-competent viral particles (Escors and Breckpot 2010). With this approach, negligible activation of T cells and natural killer cells was observed after in vivo transplantation of the engineered HILOs, highlighting the importance of the expression of self-protective molecules on the surface of transplanted cells.

4.1.3 | Integration of HLA Molecule Removal and Self-Antigen Expression

The knockout of HLA Class I and II molecules has effectively reduced T-cell-mediated rejection by limiting antigen presentation (Deuse et al. 2019; Parent et al. 2021). However, the absence of HLA molecules can trigger innate immune responses, particularly from natural killer (NK) cells. As the display of self-protective molecules can promote the survival of transplanted cells, it is reasonable to integrate HLA molecule removal and

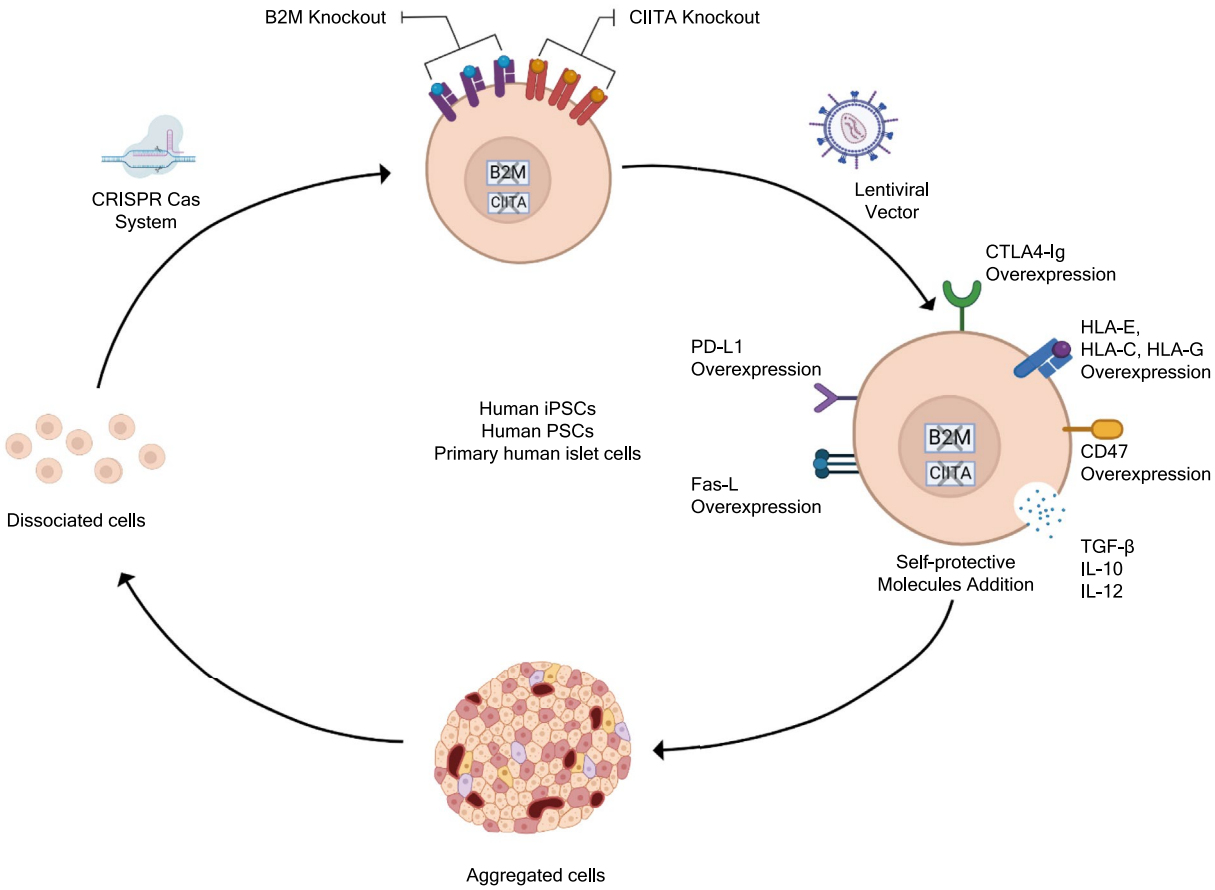

FIGURE 7 | Process of genetic engineering for the Removal and/or display of specific molecules. The CRISPR–Cas system is used to selectively disrupt B2M, CITTA, or both, enabling targeted depletion of HLA class molecules. Meanwhile, lentiviral vectors facilitate the stable integration of one or more self-protective molecules, promoting the development of immune-evasive cell populations.

the expression of self-protecting molecules for hypoimmune engineering (Figure 7).

Deuse et al. engineered a genetically modified cell line from mouse-induced pluripotent stem cells (miPSCs) to enhance immune evasion for transplantation (Deuse et al. 2019). Using CRISPR–Cas9, they knocked out HLA class I and II molecules to prevent recognition by the adaptive immune system while employing a lentiviral vector to stably overexpress CD47, a key immune-regulatory protein. CD47 binds to signal regulatory protein-α (SIRPα) on immune cells, inhibiting phagocytosis and preventing immune-mediated rejection (Deuse et al. 2019). As a result, these engineered cells elicited no antibody response and effectively evaded natural killer (NK) cell-mediated attacks, demonstrating strong potential for immune-evasive transplantation strategies.

Hu et al. applied the HIP approach to primary human islets. The engineered islets demonstrated effective immune evasion in immunocompetent animals (Hu et al. 2023; Hu, White, Olroyd, et al. 2024; Hu, White, Young, et al. 2024). Notably, these engineered cells retained an additional safety mechanism—selective clearance with anti-CD47 treatment, allowing precise immune modulation while mitigating long-term risks. This dual approach balances immune protection with controlled elimination, offering a promising strategy for transplantation therapies. Another group also knocked out both HLA class I and HLA class II genes, but uniquely retained HLA-E expression (Han et al. 2019). This approach was further differentiated by the introduction of HLA-G, CD47, and PD-L1 into the AAVS1 locus using two donor plasmids delivered via electroporation. By retaining HLA-E expression and incorporating these additional self-protective molecules, the strategy addressed vulnerabilities arising from the absence of HLA class I and II molecules. HLA-E and HLA-G provided protection against natural killer (NK) cell-mediated destruction, while CD47 inhibited macrophage phagocytosis, and PD-L1 suppressed T-cell activation, collectively enhancing immune evasion and promoting cell survival (Han et al. 2019).

Gerace et al. expanded these strategies by engineering stem cell-derived islets to overexpress PD-L1 and HLA-E, while also promoting the secretion of immunosuppressive cytokines, including IL-10 and transforming growth factor β (TGF-β; Gerace et al. 2023). Specifically, CRISPR–Cas9 was used to remove HLA molecules, plasmids targeting the GAPDH locus in human embryonic stem cells (hESCs) were introduced to drive PD-L1 and HLA-E expression, and viral vectors encoding cytokines were delivered to further enhance immune resistance and prolong islet survival in animal models.

Collectively, these studies highlight the significant progress in integrating genetic editing and self-antigen expression to address the complex immune challenges of transplantation. By combining the removal of HLA class molecules with the introduction of self-protective molecules such as PD-L1, HLA-G, and CD47, and incorporating additional layers of immune modulation (e.g., cytokine secretion), researchers have developed innovative strategies to enhance graft survival. While these approaches have demonstrated great promise in animal models, further research is required to refine these techniques, ensure long-term safety and efficacy, and translate these advancements into clinically viable solutions for transplantation.

4.1.4 | Microenvironment Engineering

Modifying the transplant microenvironment is a promising approach to prevent immunological rejection of islets. This strategy involves a variety of techniques, including the use of biomaterials, accessory cells, and engineered molecules to create a local environment that supports islet survival and limits immune activation.

One technique involves co-transplanting accessory cells, such as mesenchymal stromal cells (MSCs), to create an immunomodulatory environment. Wang et al. demonstrated this approach by genetically engineering MSCs to overexpress programmed death ligand 1 (PD-L1) and cytotoxic T lymphocyte antigen 4 immunoglobulin (CTLA4-Ig) using a lentiviral vector carrying the genes for PD-L1 and CTLA4-Ig (Wang et al. 2022). CTLA4-Ig inhibits T-cell activation through the blockade of the CD28-B7 co-stimulatory signal, effectively disrupting the immune activation cascade (Figure 8a). Together, these modifications reduced T-cell activity and protected transplanted islets from immune-mediated rejection, significantly extending graft survival in both syngeneic and allogeneic animal models. However, the engineered MSCs failed to promote long-term islet engraftment.

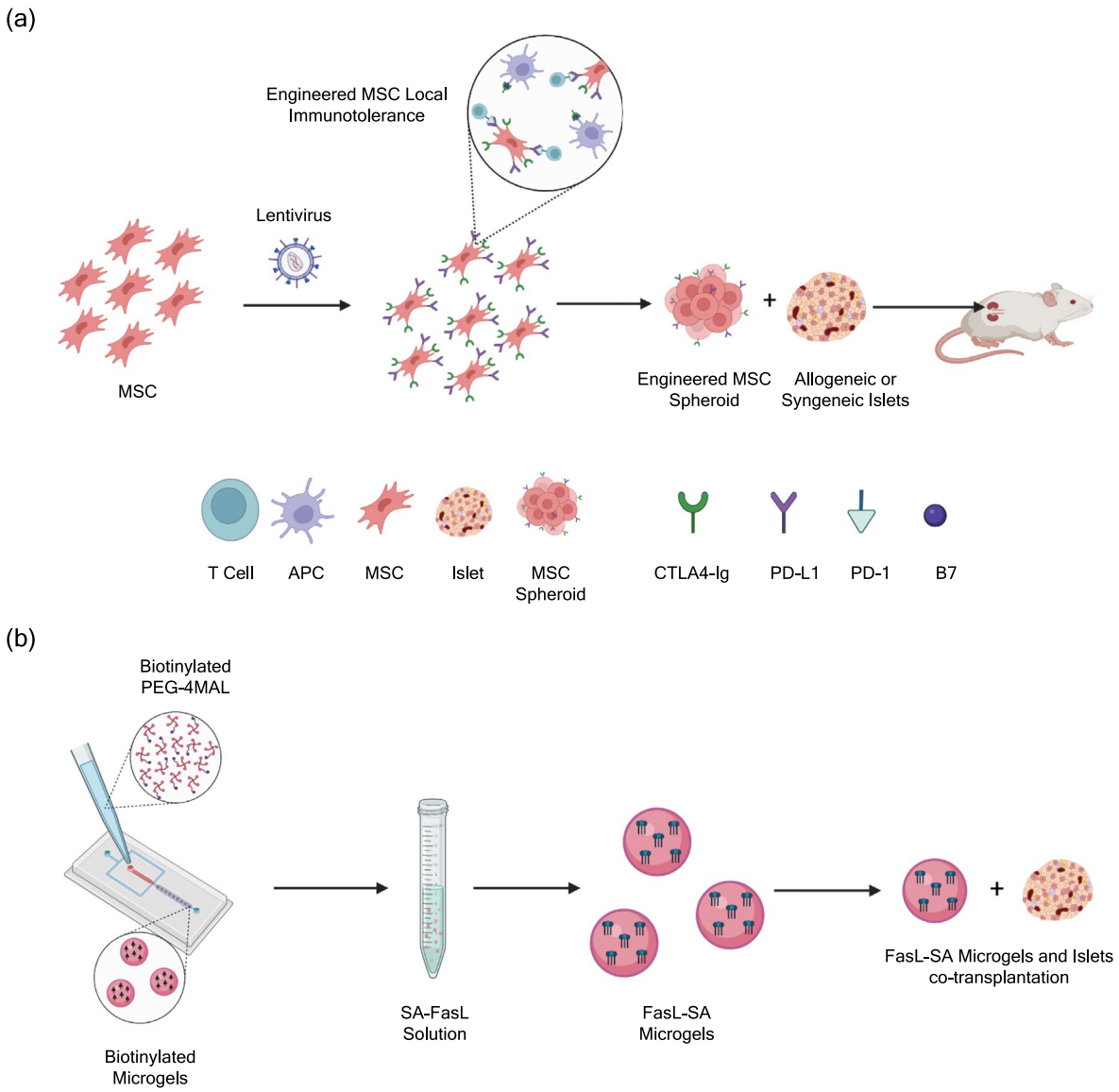

FIGURE 8 | Microenvironmental engineering strategies for immune evasion. (a) Genetically engineered MSCs overexpressing immunomodulatory molecules, including CTLA4-Ig and PD-L1, to establish an immune-privileged microenvironment. (b) Development of immunomodulatory microgels with FasL constructs to induce localized immune suppression and attenuate allogeneic immune rejection.

In addition to using accessory cells, biomaterial-based approaches have been explored to mitigate allorejection. Headen et al. developed hydrogel microgels composed of maleimide-terminated four-arm PEG (PEG-4MAL) functionalized with biotin–PEG–thiol to create biotinylated microgels (Headen et al. 2018). These microgels were crosslinked and formed in microfluidics, where the maleimide groups on PEG-4MAL react with dithiothreitol to form a stable crosslinked network, resulting in microgels with a uniform diameter of 150 μm. The biotinylated microgels were then incubated with a SA-FasL fusion protein, facilitating the display of FasL on their surface through the biotin–streptavidin interaction (Figure 8b). While this biomaterial-based platform required a short course of rapamycin, it effectively demonstrated that local immunomodulation supports islet survival by inducing apoptosis in alloreactive immune cells.

4.2 | In Vivo Studies

4.2.1 | Animal Studies

Numerous hypoimmune-engineered cells were tested in animal models to evaluate the effects of hypoimmune engineering on immune rejection and long-term cell survival without immunosuppressants. Parent et al. engineered human pluripotent stem cells (hPSCs) by deleting HLA-A, HLA-B, and HLA-C while selectively retaining HLA-A2 (Parent et al. 2021). In vivo results demonstrated significantly improved survival of HLA-A2R grafts compared to wild-type controls, with differences becoming evident as early as 4 weeks post-transplantation. HLA-A2R β-like cells survived for up to 16 weeks, underscoring their reduced immunogenicity. Notably, the absence of natural killer (NK) cells 4 weeks after transplantation alleviated concerns regarding NK-mediated rejection due to HLA class I deletion. PD-L1-expressing human islet-like organoids (wHILOs) were also studied in diabetic mice (Yoshihara et al. 2020). wHILOs successfully restored and maintained glycemic control for over 50 days. Flow cytometry of cells recovered at day 27 revealed a significant reduction in CD45+ immune cells and natural killer (NK) cells surrounding the transplanted wHILOs, indicating an immunosuppressive effect on immune cells. wHILOs were further transplanted into a reconstituted human T-cell diabetic mouse model to evaluate their therapeutic efficacy. Glycemic control was achieved and hyperglycemia returned upon wHILO removal, which demonstrates the critical role of PD-L1 expression in providing immune protection and maintaining the ability of the cells in glucose regulation.

In contrast to these genetically engineered cells, SA-FasL-engineered BALB/c islets were found to survive significantly longer in immunocompetent diabetic C57BL/6 mice, with 18% of grafts surviving beyond 100 days post-transplantation without the need for immunosuppression (Yolcu et al. 2011). Even more strikingly, transient administration of rapamycin extended graft survival for over 500 days. Immunohistochemistry revealed a significant increase in Treg cells by day 5 post-transplantation. These cells were even observed near the islets at 100 days, which emphasizes their role in promoting immune tolerance. Meanwhile, no evidence of neutrophil influx was observed, demonstrating the safety and immunomodulatory properties of SA-FasL-engineered islets. However, SA-PD-L1-engineered islets were rejected in the absence of immunosuppression within 28 days, and a 15-day rapamycin regimen extended graft survival to 100 days (Batra et al. 2020). The islets maintained normal glucose levels, with hyperglycemia returning upon graft removal, confirming their regulatory role. Additionally, Treg depletion using diphtheria toxin led to graft rejection, highlighting the critical role of Tregs recruited by the engineered islets in maintaining immune tolerance.

Combining HLA class molecule knockout with the overexpression of immunomodulatory molecules was also studied in animals. Genetically engineered mouse iPSCs (miPSCs) without HLA class I and class II molecules, while overexpressing CD47, were transplanted into allogeneic BALB/c mice without using immunosuppressants (Deuse et al. 2019). All the engineered cell lines formed teratomas, which are indicative of retained pluripotency, but did not induce T-cell, NK-cell, or antibody-mediated immune responses. Transplanted miPSCs exhibited long-term survival when transplanted into both syngeneic and allogeneic mice due to their resistance to immune clearance. Using a similar approach, HIP-engineered islets were developed by eliminating HLA class I and II expression and overexpressing CD47, and their functionality was evaluated in diabetic cynomolgus monkeys and humanized mouse models (Hu et al. 2023; Hu, White, Olroyd, et al. 2024; Hu, White, Young, et al. 2024). These engineered islets restored the normoglycemia of cynomolgus monkeys within 12 days, maintaining glycemic control for 6 months without immunosuppression (Hu, White, Young, et al. 2024). Immunological analyses revealed no T-cell or NK-cell activation targeting the HIP islets. The role of CD47 in maintaining glycemic control was confirmed by graft destruction using an anti-CD47 IgG4 antibody, as hyperglycemia returned. In humanized mouse models, HIP-engineered islets achieved glycemic control, even when partially nonengineered islets were present, showcasing their robustness (Hu et al. 2023).

Multiple immune cells play important roles in mediating cell rejection. Thus, the addition of multiple self-protecting molecules to suppress different types of immune cells was also used for hypoimmune cell engineering. hPSCs modified with the addition of HLA-E, HLA-G, CD47, and PD-L1, along with the removal of HLA class I and II, were transplanted in vivo (Han et al. 2019). Immunodeficient mice were chosen to facilitate teratoma formation over a 4- to 6-week period and to enable the adoptive transfer of presensitized allogeneic $CD8^+$ T cells, which were activated, as indicated by $CD69^+$ expression, without showing signs of PD-1-mediated exhaustion. Upon $CD8^+$ T-cell injection, the resulting teratomas were larger than wild-type teratomas, suggesting reduced immune rejection. qPCR analysis revealed reduced T-cell infiltration, with lower expression of CD8 and IL-2 markers in the hPSC teratomas. These findings suggest that incorporating multiple self-protecting molecules, in addition to removing HLA molecules, is beneficial in preventing T-cell-mediated rejection. In addition to surface modifications, cells can be engineered to release specific cytokines. Engineered islets that overexpressed PD-L1 and HLA-E, alongside cytokines such as IL-2, IL-10, and TGF-β, were transplanted into mice. These cells survived up to 9 weeks post-transplantation, while wild-type grafts were rejected within 2 weeks (Gerace et al. 2023). In autoimmune diabetic NOD mice, the engineered islets could reverse diabetes for 8 weeks post-transplantation. The presence

of Tregs within the grafts underscored the immunosuppressive and functional efficacy of these hypoimmune-engineered islets.

Beyond engineering the islets themselves, alternative strategies have explored the use of accessory cells and biomaterials to modulate the local immune environment and further support graft longevity. MSCs were transplanted into C57BL/6 mice to assess their ability to prolong islet graft survival (Wang et al. 2022). Without MSCs or with nonengineered MSCs, transplanted islets were rejected within 14–20 days. In contrast, after mice received islets co-transplanted with PD-L1/CTLA4-Ig-expressing MSCs, they could maintain normal glucose levels for 40 days, with some lasting up to 100 days. Immunological profiling revealed fewer CD4+ and CD8+ T effector cells in the transplantation area and a higher presence of Tregs. In contrast, when engineered MSCs were transplanted in one kidney and islets in the other, the islets were rejected within 14 days. These observations suggest that the engineered MSCs fostered a localized immune-tolerant environment, underscoring the importance of co-localization for their immunomodulatory effects. Immunomodulatory SA-FasL-expressing microgels were also explored for islet transplantation in treating diabetic mice (Headen et al. 2018). Islets co-transplanted with SA-FasL microgels survived an average of 31 days, with 25% surviving beyond 200 days, compared to 15 days for islets with FasL-free microgels. When combined with a short 15-dose course of rapamycin, a high percentage of grafts remained functional in the kidney capsule for over 200 days. However, when allogenic islets were co-transplanted with SA-FasL microgels in the epididymal fat pad, the survival rate significantly decreased, with 50% lost within 30 days, likely due to differences in immune responses and suboptimal immunomodulatory dosing at the transplantation site. This study suggests that while further optimization is needed to enhance islet survival in the epididymal fat pad, immunomodulatory microgels remain a promising method for islet co-transplantation.

4.2.2 | Clinical Trials

VX-880 is an hiPSC-derived, fully differentiated insulin-secreting cell therapy by Vertex Pharmaceuticals (CRISPR Therapeutics AG 2023). This therapy is designed to functionally replicate human islets, offering a renewable source of insulin-producing cells for patients with T1D. Early-phase clinical trials demonstrated successful islet engraftment and glucose-responsive insulin secretion in all 12 enrolled patients within 90 days, and the patients exhibited improved glycemic control with mild to moderate adverse events (CRISPR Therapeutics AG 2023; Vertex Therapeutics 2025). While these results highlight the potential of stem cell-derived islet replacement therapy, the patients need long-term drug regimens to prevent immune rejection. The continued requirement for chronic immunosuppression still presents a major challenge, underscoring the need for developing genetically modified cells with hypoimmune engineering methods that can evade immune detection and eliminate the dependency on lifelong immunosuppression. CRISPR Therapeutics is conducting a phase 1/2 clinical trial for VCTX211, a combination therapy that incorporates genetically modified allogeneic pancreatic endoderm cells to confer immune evasion in T1D (Vertex Pharmaceuticals Incorporated 2023). VCTX211 leverages CRISPR–Cas9 technology to introduce genetic modifications that reduce immune recognition, potentially allowing for long-term graft survival without immunosuppressive drugs. It targets B2M (a component of MHC Class I molecules) and CITTA, both of which play a role in immune recognition. By modifying these molecular targets, VCTX-211 seeks to make transplanted islet cells less detectable by the immune system. While no updated clinical findings have been reported, this trial represents an important step in translating hypoimmune engineering strategies into human applications.

Notably, preclinical studies leveraging CRISPR–Cas9 and lentiviral vector approaches have demonstrated encouraging results in immune evasion and graft survival, demonstrating the potential for future clinical applications. However, genetic modifications—such as PD-L1, CTLA4-Ig, and CD47 overexpression, along with HLA deletion—have yet to be fully evaluated in clinical trials. If successful, the integration of these hypoimmune strategies could expand islet transplantation eligibility to populations currently excluded due to the risks of immunosuppressive therapies, including children and patients with coexisting health conditions. Additionally, hypoimmune engineering offers a promising solution to donor shortages by facilitating the development of universal donor islets.

4.2.3 | Challenges and Perspectives

Advances in chemical conjugation and functionalization have significantly improved the biocompatibility of cell microencapsulation and nanoencapsulation materials. Resultantly, fibrosis and inflammation at transplantation sites have been minimized, which enhances the overall success of islet transplantation. However, methods for microencapsulation and nanoencapsulation still face multiple challenges.

Most encapsulation methods rely on syringe-based or microfluidic devices, which have fixed channel geometries incompatible with the size variability of islets isolated from the pancreas. This mismatch can cause occlusions and clogging, creating inefficiencies in large-scale manufacturing. Certainly, increasing the channel size would help mitigate this issue, but it will cause a problem with increasing microcapsule size and transplantation volume. The large size of capsules exacerbates diffusion limitations, restricting oxygen and nutrient transport and ultimately leading to hypoxia-induced islet dysfunction. The large transplantation volume also limits the ability of an organ to house a sufficient number of islets for effective treatment. This issue will be worsened for patients who need multiple transplantations. Additionally, the stochastic nature of droplet formation results in capsules that contain no islets or a varied number of islets, further increasing transplantation volume and reducing therapeutic consistency. Device-free encapsulation methods, including nanoencapsulation, may help solve many of these problems, such as reducing the size of capsules. However, these methods often require the involvement of harsh conditions, including high shear stress, organic solvent, low pH, and free radicals, all of which can damage islets, making the process challenging for clinical translation. Excessive reduction in capsule size may lead to coating defects and compromised mechanical stability, which could be one of the reasons why nanoencapsulation methods

have not been studied in any clinical trials. Furthermore, immune cells may extend their cell membranes to penetrate thin capsules due to their dynamic membrane nature, ultimately recognizing and eliminating the encapsulated islets. Therefore, it is essential to develop innovative methods that integrate the advantages of existing encapsulation techniques. An ideal method would be device-free, stress-free, and free from complex procedures, ensuring a uniform coating with an optimal capsule thickness—neither too thick nor too thin.

In addition to the ongoing search for novel encapsulation methods, further efforts are needed in the development of advanced biomaterials. The incorporation of zwitterionic chains has notably enhanced the nonfouling properties and biocompatibility of encapsulation materials. However, a key challenge remains since islet cells naturally reside within a basement membrane that supports cell attachment. While nonfouling materials help prevent fibrotic overgrowth, they may also hinder cell adhesion, potentially compromising islet cell survival and function. Addressing this trade-off is crucial for achieving long-term cell viability and therapeutic efficacy. Furthermore, encapsulation materials must offer sufficient stability to protect the cells during diabetes treatment. At the same time, strategies for removing or degrading these materials after cell death remain underdeveloped. Certainly, an alternative strategy is to minimize the volume of encapsulating material. This approach could reduce the need for material removal, and ideally, the materials would either degrade naturally or be easily retrieved by the time most encapsulated cells have died. It is also important to emphasize that material development and method innovation must be compatible. An ideal encapsulation method should avoid harsh conditions—such as low pH or the generation of free radicals—that can cause short- or long-term cellular damage. Striking a balance between material performance and gentle manufacturing processes is essential for developing effective and scalable encapsulation technologies.

Hypoimmune engineering presents a promising approach to islet transplantation by enabling immune evasion without the need for encapsulation or chronic immunosuppression. Co-transplanting accessory cells or supportive materials that attenuate active immune cells—either by releasing cytokines or displaying inhibitory ligands—has been shown to significantly extend the survival of transplanted islets. However, the lifespan of accessory cells or the stability of supportive materials may be shorter than the duration required for islet function. Once these supporting components degrade or disappear, the transplanted islets may lose their protective shield, potentially compromising their long-term viability. Gene editing and delivery methods offer a strategy to functionalize the surface of islets for immune evasion without relying on encapsulation or co-transplantation of accessory cells or supportive materials. However, this approach requires islets to undergo iterative cycles of cell assembly and disassembly due to multiple rounds of gene manipulation or delivery. The generation of pigs that either express human transgenes to enhance compatibility or lack genes responsible for xenogeneic immune responses represents a promising approach for organ and cell transplantation. However, this strategy demands advanced genetic engineering techniques, along with specialized housing and breeding facilities, which present substantial logistical and financial challenges. The impact of this process on long-term cell function and reliability remains to be fully validated. Additionally, the complexity and time-intensive nature of these protocols pose challenges to the scalability of islet manufacturing and the feasibility of widespread clinical application. Despite these concerns, it is important to recognize that hypoimmune engineering is still in its early stages of development. A rational integration of various hypoimmune engineering strategies may offer innovative solutions to overcome current challenges.

Author Contributions

Kyungsene Lee: writing – original draft (lead), writing – review and editing (supporting). **Ana Aviles Vargas:** writing – original draft (supporting), writing – review and editing (supporting). **Rita Bottino:** writing – review and editing (supporting). **Yong Wang:** conceptualization (lead), funding acquisition (lead), project administration (lead), supervision (lead), writing – review and editing (lead).

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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