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On the path to a diabetes cure: a critical appraisal of developments in pancreatic beta cell encapsulation and implantation

En el camino hacia una cura de la diabetes: una evaluación crítica de los desarrollos en la encapsulación e implantación de células beta pancreáticas

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Abstract

Microencapsulation of β-cells is a rapidly growing field that offers broad potential for the therapy and possible cure of diabetes, especially type 1 diabetes, thanks to the immunization of the engrafted tissue that increases its long-term efficacy and decreases the risk of immunogenicity. Despite the promising results obtained in human and animal studies, important challenges need to be addressed. The structure and composition of the microspheres and the site where they are implanted can affect the effectiveness of the treatment, and associated immunogenicity problems have been reported. To improve the safety of the encapsulated islet graft system, new efforts are being made in the bioengineering of the capsules and the production of insulin-producing cells within the capsular membranes. These critical advances in cell encapsulation technology are expected to enable broader and more effective human application of this system. In this review, the great potential of encapsulated pancreatic islet transplantation to provide a cure for type 1 diabetes is highlighted. The advantages and disadvantages of this therapeutic strategy are also outlined, as well as key advances made in cellular microencapsulation research for treating diabetes.

Keywords: Type 1 Diabetes, Microencapsulation, Insulin

Resumen

La microencapsulación de células β es un campo en rápido crecimiento que ofrece un amplio potencial para la terapia y posible cura de la diabetes, especialmente la diabetes tipo 1, gracias a la inmunización del tejido injertado que aumenta su eficacia a largo plazo y disminuye el riesgo de inmunogenicidad. A pesar de los resultados prometedores obtenidos en estudios en humanos y animales, es necesario abordar importantes desafíos. La estructura y composición de las microesferas y el sitio donde se implantan pueden afectar la eficacia del tratamiento y se han reportado problemas de inmunogenicidad asociados. Para mejorar la seguridad del sistema de injerto de islotes encapsulados, se están realizando nuevos esfuerzos en la bioingeniería de las cápsulas y la producción de células productoras de insulina dentro de las membranas capsulares. Se espera que estos avances críticos en la tecnología de encapsulación celular permitan una aplicación humana más amplia y efectiva de este sistema. En esta revisión, se destaca el gran potencial del trasplante de islotes pancreáticos encapsulados para proporcionar una cura para la diabetes tipo 1. También se describen las ventajas y desventajas de esta estrategia terapéutica, así como los avances clave realizados en la investigación de microencapsulación celular para el tratamiento de la diabetes.

Palabras clave: Diabetes tipo 1, Microencapsulación, Insulina

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Introducción

Diabetes mellitus (DM) is a growing cause of global morbidity and mortality. More than half a billion people are living with diabetes worldwide, affecting men, women, and children of all ages in every country. That number is projected to more than double to 1.3 billion people in the next 30 years, with every country seeing an increase (GBD 2021 Diabetes Collaborators, 2023). In 2014, its prevalence was around 442 million people (Lovic et al., 2020), increasing to over 536.6 million in 2021, with estimates exceeding 783.2 million individuals expected to suffer from this disease by 2045 (Sun et al., 2022; Paneni et al., 2013). DM is strongly associated with comorbidities such as obesity, hypertension, and dyslipidemia. Furthermore, this disease is associated with a high number of macrovascular and microvascular complications, such as ischemic heart disease affecting 10% of diabetic individuals, diabetic nephropathy with a prevalence of up to 35%, and diabetic retinopathy, which is the leading cause of blindness in diabetic patients affecting between 12% and 18% of them (Aikaeli et al., 2022). Consequently, there is a constant demand for the development of therapies that prevent and effectively control these disorders and save lives (Armengol et al., 2021).

The continuous loss of pancreatic β-cells with the subsequent deficiency in insulin production is the hallmark of type 1 diabetes (T1D) and late-stage type 2 diabetes (T2D) (Alejandro et al., 2015; Atkinson et al., 2014). The current standard treatment for this subgroup of patients is the administration of human recombinant insulin and insulin analogs through the classic subcutaneous and intravenous routes, the recently developed inhaled route, and the routes currently under development that will be available in the near future, such as the transdermal route and oral insulin.

These therapies include different types of insulin, such as long-acting insulin, which provides the basal insulin component normally combined with rapid—or ultra-rapid-acting insulin administered before meals. In this way, it is sought to mimic pancreatic physiological function and thus achieve better results (American Diabetes Association Professional Practice Committee, 2022).

However, despite being the "gold standard" in the treatment of T1D and T2D with advanced beta secretory failure, exogenous insulin therapy has been associated with adverse effects such as weight gain, lipoatrophy, hyperlipotrophy, skin infections, hypoglycemia (Demir et al., 2022; Guarneri and Hoffman, 2022; Amiel, 2021), and even therapeutic failure in a considerable percentage of patients (Al-Qerem et al., 2022; Sherwood et al., 2020; Shetty and Ramachandrappa, 2019; Alzaheb and Altemani, 2018). As a result, efficient therapeutic strategies have been developed in recent decades to effectively control these disorders and restore endogenous insulin production (Posselt et al., 2010).

An existing approach, different from conventional diabetes treatments, is the transplantation of human cadaveric islets to replace the patient's destroyed β-cells. This procedure generally results in improved glycemic control, rendering patients insulin-independent for extended periods and improving overall quality of life (White et al., 2009). While a promising approach, this therapy presents certain limitations, such as a shortage of cadaveric donors

compared to the large number of diabetic patients requiring the intervention, the need for chronic immunosuppression to prevent graft rejection, and complications related to the immunological component of DM (Shapiro, 2000 and 2011).

As a result of these limitations, new immune-invasive alternatives have emerged, such as the microencapsulation of pancreatic islets. This technique represents a promising approach to ensuring the viability and function of transplanted cells (Pereira et al., 2020). Encapsulating these cells in a biocompatible material reduces the probability of immune rejection reactions while extending their half-life without affecting their endocrine function. This opens up new possibilities for xenotransplantation, the transplantation of non-human cells of animal origin, such as porcine cells, increasing the availability and reducing the cost of these cells (Safley et al., 2018; Shimoda and Matsumoto, 2017).

Cellular microencapsulation techniques

Cell encapsulation is a technology mainly implemented in cell therapies and tissue bioengineering (Pereira et al., 2020). This is because it allows for the rapid and efficient reproduction of the natural microenvironment and enables the proper organization of immobilized cells (Bidoret et al., 2017). This technology is based on the immune isolation of cells or cell clusters suspended in a biocompatible substance. These cells are protected by an outer protective layer that maintains their structural integrity and allows optimal nutrient exchange for survival and proliferation (Calafiore et al., 2017).

Encapsulation strategies have traditionally been categorized into two groups based on the length scale of the encapsulation structure: microencapsulation and macroencapsulation. While cell attachment is generally required for the survival of encapsulated cells, in some microencapsulation applications, such as those aimed at tissue repair or regeneration, biodegradability of the protective layer may be desirable. When this layer degrades, the trapped cells can proliferate and create their own extracellular matrix instead of the artificial one used to entrap them (Opara, 2017a,b).

Microencapsulation consists of three main stages. First, the cells are mixed with the substance that will be the main component of the capsule, where they will be suspended (usually a liquid). Second, they are dispersed into droplets. Finally, they are converted into microspheres (Bidoret et al., 2017). There are different methods for manufacturing microcapsules, such as emulsification, dispersion, and solvent evaporation. However, most of these methods use organic solvents and high vortex speeds, which have high cytotoxicity and make it impossible to create microcapsules (Imaninezhad et al., 2019). One of the most common and safe methods is using coaxial air jets (Walker et al., 2015; Hsu and Fu, 2017).

Another widely used method is fluid extrusion, in which a solution of cells suspended in a liquid is taken up in glass syringes and pumped into microchannels using syringe pumps to generate droplets. Depending on the number of flow capillaries, droplets can be generated in three regimes: squeezing, dripping, and jetting, using a flow-focusing junction (Jayaprakash and Sen, 2019).

Recent studies have shown increasing interest in developing and implementing new techniques for creating microcapsules capable of containing live cells. One of the most promising techniques is electrohydrodynamic spraying (EHDS) (Gansau et al., 2018). EHDS has an excellent ability to fabricate microspheres with a wide range of sizes between 70-700 μm (Imaninezhad et al., 2019).

EHDS creates microspheres by pumping a liquid through a fine metal capillary and applying a high electrical voltage between the capillary and a ground collector. The driven liquid overcomes surface tension forces by accumulating opposite charges on its surface, which occurs if a critical applied voltage (Vcr) is exceeded. Further increase in the applied voltage leads to a conical liquid meniscus at the needle tip called the Taylor cone, which causes a continuous jet that breaks up into fine droplets on the surface of a collector. To achieve cell encapsulation with this technique, a suspension of live cells is pre-mixed with the precursor liquid and sprayed by applying a high voltage as described above. The microdroplets are then collected in a collector medium that induces their transition to a gel, allowing cell encapsulation within (Qayyum et al., 2017).

Structure

The main determinant factor in the success of cell-based therapies lies in the composition of the device in which they are encapsulated. This device must be a biocompatible polymer envelope with the properties of a semipermeable barrier. It must not trigger autoimmune responses that endanger the recipient's integrity or the cells inside the device (Bose et al., 2020).

Despite the wide availability of polymers used in different areas of bioengineering, very few meet the necessary properties of biocompatibility and efficiency in microencapsulation processes for cell therapies (Calafiore, 2018). In this context, two substances have become relevant and are the most used in this field: alginate and polyethylene glycol (PEG) (Aijaz et al., 2017) (Table I).

Alginate is a unique and versatile polysaccharide derived from a species known as brown algae. It comprises linear copolymers of two acidic units, β-D-mannuronic (M) and α-L-guluronic (G), repeated throughout the molecule as dimeric units GG, MM or MG. Alginate can form hydrogels in divalent cationic aqueous solutions (Ca^{2+}, Ba^{2+}) (Calafiore et al., 2017). The main drawback of alginate is its lack of selective permeability, which is crucial for adequately isolating its contents. For this reason, it is often combined with a layer of amino acid polymers, mainly poly-L-lysine (PLL) and poly-L-ornithine (PLO) (Kendall Jr and Opara, 2017) (Figure 1).

On the other hand, PEG encapsulation is an alternative to alginate. Its use lies in modifying the cell surface by conjugating PEG, a process known as PEGylation. This procedure is carried out using heterofunctional PEG: NHSPEG-CH3 (NHS-mPEG). The N-hydroxysuccinimide (NHS) group allows spontaneous reactivity with free amines, while the methyl (CH3-) group leaves an inert terminal end (Giraldo et al., 2017). Compared to alginate, PEGylation improves cell viability by reducing the diffusion barrier and enabling transplantation via the portal vein (Park et al., 2018). However, PEGylation does not entirely prevent the rejection of allogeneic and xenogeneic grafts, likely due to the limited capacity of individual PEG

Table I.
A list of capsules reported in the literature for islet encapsulation and transplantation

Type	Materials	Encapsulation method	Design advantage
Photo-crosslinked	• 4-armed PEG cystine • Laminin and laminin peptides	• Islet suspension in hydrogel solution and photopolymerization	• Increased insulin production • Prevention of fibroblast attachment
Ultra-thin layers	• PEG	• Layer-by-layer PEG application	• Thin membrane increased small molecule diffusion
Thin layers	• PVPON and TA	• PVPON adsorption to islet surface followed by TA absorption	• Created thin layers using PVPON • Used TA to reduce the ROS
Cell-based capsule	• PEG-lipid-biotin layer • HEK293 cells	• Islets coated with PEG-lipid-biotin layer • Coated islets were cultured with streptavidin-immobilized HEK293 cells, and a biotin streptavidin reaction resulted in HEK293 cells covering the surface, forming a capsule	• Potential to improve immunoisolation due to capsule made of cells
ECM-based microcapsule	• Fibronectin • Gelatin	• Layer-by-layer coating	• ECM-mimicking capsule materials created a more realistic microenvironment • Greater islet insulin secretion
Ion-crosslinked microcapsule	• High-mannuronic acid alginate	• Air droplet generation crosslinked with $BaCl_2$	• Increased control over droplet size
Ion-crosslinked microcapsule	• Alginate	• Electrostatic droplet generation crosslinked with $BaCl_2$	• Smaller microcapsules compared with air-based droplet generation
Core-shell microcapsule	• Alginate • Matrigel	• Co-axial electrostatic droplet generation gelled using $BaCl_2$	• Islets were contained in the center of the capsule, reducing fouling • Core material can be modified to improve islet survival
Conformal coating	• PEG • Alginate coating	• Droplet generation using small needle • Capsules crosslinked using DTT	• Thin membrane around capsule using droplet-based generation
Crosslinked on-chip capsule	• PEG	• On-chip flow focusing microcapsule creation, crosslinked with DTT	• Smaller capsule diameter size
High-throughput ion-cross-linked micro-capsules	• Alginate	• On-chip nozzle capsule formation, crosslinked in $CaCl_2$ gelling bath	• High throughput design with eight channels for faster capsule generation

Source: White et al., 2020

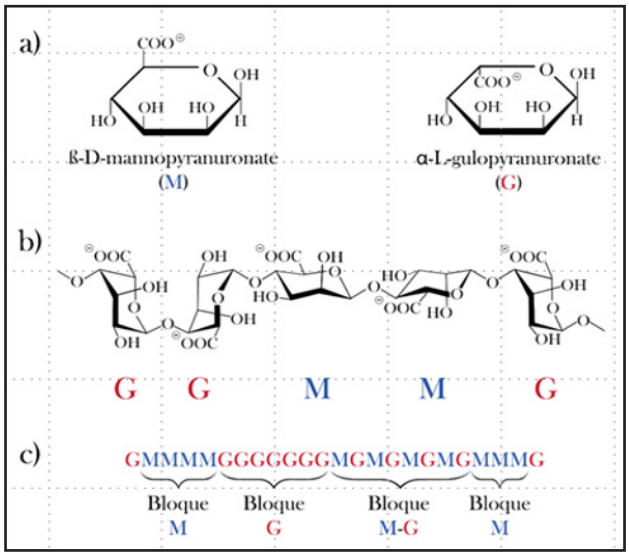

Figure 1. Alginate Structure. Alginic acid is a polysaccharide composed of two types of uronic acid: Mannuronic acid unit (M) and Guluronic acid unit (G), which form three kinds of polymer segments of blocks. (a) Alginate Monomers (M and G), (b) Macromolecular, (c) Block Sequence of M, G, and M-G Units

chains to provide durable protection against aggressive immune responses. Therefore, PEG-based cell therapies are often combined with local immunosuppression to ensure greater benefits (Stabler et al., 2020) (Figure 2).

Therapeutic Limitations

Nanoencapsulation is a developing technology with great potential, although it has certain limitations that depend on the structure and composition of the microspheres and the site of implantation. These limitations must be overcome to ensure the reliability and safety of microencapsulated pancreatic beta-cell transplantation (Opara, 2017 a,b).

Structural Instability

Membrane stability is a major challenge in microencapsulation that limits its clinical application due to the cracking of the polymeric membrane of the materials used to create microspheres (Toda et al., 2019). On the one hand, alginate is susceptible to swelling and subsequent rupture after transplantation due to osmotic stress, which leads to loss of immunoisolation and graft rejection. Furthermore, the membrane stability of PEG-based microcapsules is lower than that of hydrogel-based capsules because their uniformity is still poor, which causes them to break. However, recent studies have shown a significant improvement in PEG capsules, which are more resistant to osmotic stress than alginate (Park et al., 2018).

A promising option is using hybrid alginate-PEG microspheres, which are less prone to osmotic swelling and provide greater stability after exposure to chelating agents than alginate or PEG spheres alone (Köllmer et al., 2016).

Immune Response

Despite the great potential of microencapsulated cell-based therapy, there are still multiple reasons why no clinically licensed therapeutic product based on this technology has yet reached the market. The main reason is the challenge of counteracting the host immune response caused by both the implanted capsule and the encapsulated cells (Ashimova et al., 2019). Therefore, it is necessary to use the most purified polymers possible since, for example, commercially available crude alginates contain pro-inflammatory pathogen-associated molecular patterns (PAMPs), such as flagellin, lipopolysaccharide, peptidoglycan, lipoteichoic acid, and polyphenols, which induce inflammatory responses in receptors either by diffusion out of the capsules or by being present in the capsule. Likewise, the presence of PAMPs has been evidenced in

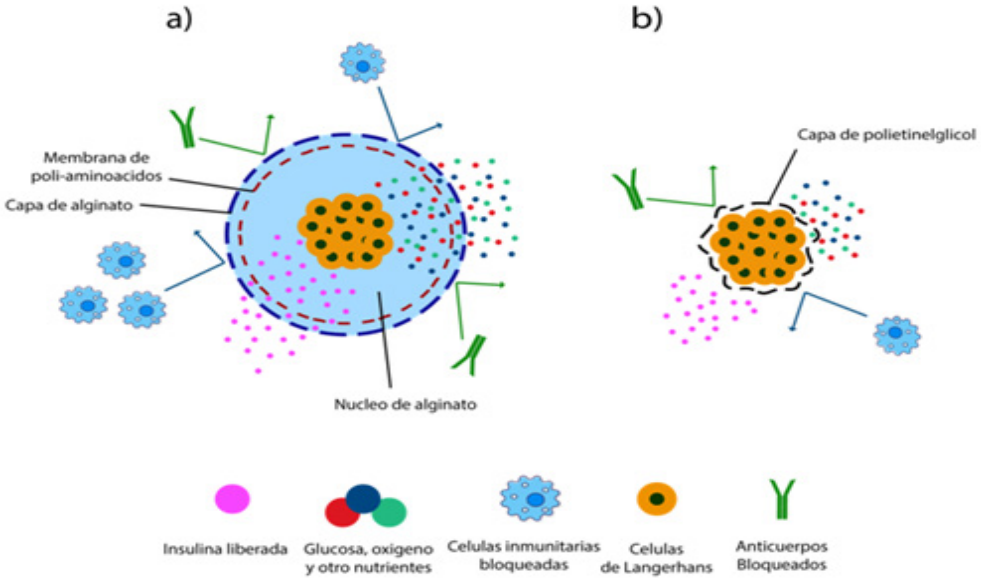

Figure 2. Microcapsule Structure. Schematic illustration of the structure of microcapsules. a) Alginate-based microcapsules with a longer diffusion distance. These are composed of linear copolymers of two acid units, β-D-mannuronic (M) and α-L-guluronic (G), repeated throughout the molecule as dimeric units. b) A Polyethylene glycol (PEG)-based microcapsule with a shorter diffusion distance. This involves modifying the cell surface by conjugating PEG with it, a process known as PEGylation. Both microcapsules block the entry of immune cells and antibodies while allowing the entry of glucose, oxygen, and other nutrients and the exit of insulin released by the Langerhans β cells.

other sources, such as synthetic molecules, i.e., PEG (Hu and de Vos, 2019).

These PAMPs stimulate a cascade of signal transduction pathways, starting with Toll-like receptors (TLRs) and pattern recognition receptors (PRRs). After the activation of PRRs in immune cells, a cascade of intracellular signaling pathways is activated, leading to the translocation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) that induces the secretion of inflammatory cytokines, which ultimately results in overgrowth of the capsules by immune cells and fibroblasts (Fang et al., 2017), destroying the transplanted cells and decreasing the transport of oxygen and nutrients through the membrane, this is called foreign body response (FBR) (Korsgren, 2017).

Implantation site of microencapsulated pancreatic beta-cell transplantation

The implant site exerts an important influence on the engraftment, stability, and biocompatibility of implanted microencapsulated pancreatic beta-cells. Currently, an optimal site for encapsulated islet transplantation may include sufficient capacity to host large graft volumes, portal drainage, ease of access using a safe and reproducible procedure, adequate blood/oxygen supply, minimal immune/inflammatory reaction, pliable for noninvasive imaging and biopsy, and potential of local microenvironment manipulation or bioengineering. Varying degrees of success have been confirmed with the utilization of liver or extrahepatic sites in an experimental or preclinical

setting. However, the ideal implant site remains to be further engineered or selected for the widespread application of encapsulated islet transplantation.

The infusion of islets into the vascular system is technically straightforward, as percutaneous access to nearly any large vessel is possible with fluoroscopic guidance. Refinements have included the selection of arterial or venous locations that confer better oxygen tension and those at relatively low risk of embolic disease. The bloodstream, the uncertain distribution of the transplanted microspheres, and the stacking of capsules in the pelvic cavity of bipeds result in aggregation and difficulty in retrieval (Papas et al., 2019). On the other hand, the renal subcapsule is another option. Unfortunately, the kidney capsule has a relatively poor blood supply and does not provide an oxygen-rich microenvironment for the islets. The kidney cortex has approximately half the blood flow of the kidney parenchyma, and its oxygen tension is only 15 mmHg. In addition, the high requirement of capsules in the transplant promotes the aggregation of encapsulated islets (Shimoda and Matsumoto, 2017) (Table II).

The subcutaneous space, despite its limited access to blood, can also be used to deposit a large number of encapsulated islets; however, it requires two surgical procedures, one dedicated to vascularization and then the actual transplantation (Pepper et al., 2015; Farina et al., 2017). Unlike the subcutaneous space, the omental space could be an ideal site for implanting encapsulated islets since it does not require two operations. Omental pouch sites are attractive as they allow a large implantation volume and the concurrent use of transplant devices or capsules. Furthermore, the activated omentum has an increased number of blood vessels and blood content, as well as growth and progenitor factors such as vascular endothelial growth factor, stromal cell-derived factor 1α, the chemokine receptor CXCR4 and Wilms' tumor suppressor gene. For islet transplantation, the omentum may not only provide these benefits but also offer some immune privilege. Still, the large number of islets required in these sites and limited data on long-term survival make omental pouch approaches less attractive than others (Merani et al., 2008). The long-term function of encapsulated islets in the omental bursa was demonstrated using

Table II. Capsule Types and Materials

	Prevention of Aggregation	Close Access to Blood	Ease of Retrieval	Stable distribution
Renal subcapsule	NO	YES	NO	YES
Omental bursa	YES	YES	YES	YES
Intraperitoneal cavity	NO	NO	NO	NO
Subcutaneous Space	YES	NO	YES	YES
Prevascularized subcutaneous space	YES	YES	YES	YES

an immunocompetent diabetic rat model (Pareta et al., 2014) (Table II).

Recent advances

There has been growing interest in cell encapsulation technologies and therapies in recent years to overcome the current therapeutic limitations that hinder and slow their development. Limitations such as the immune rejection response to the capsule components and structural weakness could lead to rupture and release of their cellular content, leading to adverse immune reactions that can endanger the recipient's life (Toda et al., 2019). These advances focus mainly on alternative measures to ensure greater efficacy and safety in their implementation, ranging from a rethinking of the microcapsule structure by modifying the number of layers (Syed et al., 2018; Bhujbal et al., 2014), its conformation and surface (Alagpulinsa et al., 2019 a,b), to the combination of macro and microcapsules (Saenz del Burgo et al., 2018), among others.

Biocompatible Polymers

Foreign Body Reaction (FBR) is a complex biological response to implanted biomaterials that involves inflammatory events and wound-healing processes leading to fibrosis (Klopfleisch and Jung, 2017). There has been a growing interest in developing anti-inflammatory and anti-fibrotic biopolymers to mitigate this response in recent years. Alginate, the most widely used material for microcapsule creation, has been covalently modified with different chemical groups to achieve this goal. In an extensive study by Vegas et al. (2016a,b), 774 alginate recombinants were analyzed and subjected to various rigorous tests. Three alginate recombinants had promising properties: Z2-Y12, Z1-Y15, and Z1-Y19. All three modifications were triazole derivatives, suggesting that this class of molecules can modulate immune cell populations, specifically macrophages, on the surface of these materials in a way that inhibits their activation and disrupts fibrotic processes.

Microcapsules made with triazole-derived modified alginate, such as triazole–thiomorpholine dioxide (TMTD) alginate, have been shown to provide long-term glycemic correction and glucose responsiveness without immunosuppressive therapy for up to 174 days, demonstrating low susceptibility to FBR (Vegas et al., 2016 a,b).

There has also been research on the combination of different polymers (Correia et al., 2019), specifically alginate-PEG (Villa et al., 2017) and alginate-chitosan (Yang et al., 2016), to create bilayer capsules that provide effective immunization. This idea was later improved with the implementation of methoxy polyethylene glycol-succinimdyl valerate (mPEG-SVA), previously used in conjunction with immunoprotectors (Hashemi et al., 2017), now in combination with alginate and chitosan for the creation of an alginate-chitosan-mPEG-SVA multi-polymer microcapsule. This approach showed a decrease of approximately 38% in IL-2 production by lymphocytes compared to unencapsulated pancreatic cell islets. It showed no difference in insulin secretion compared to unencapsulated cells, demonstrating no adverse effects on the isolated cells (Najafikhah et al., 2018). However, efforts have been made to find alternatives to alginate, among which

PEOT/PBT300 stands out recently. Sthijns et al. (2021), indicated that PEOT/PBT300 showed the best results compared to other biomaterials, considering its properties of elasticity and resistance to breakage, unaffected gene expression related to angiogenesis, low levels of oxidative stress, expression of endogenous antioxidants, and viability and functionality of pancreatic beta and alpha cells. Thus, PEOT/PBT300 was demonstrated to be a promising polymer for cell encapsulation.

Toroidal Microcapsules

Pancreatic cell encapsulation and transplantation technologies and therapies offer an innovative perspective in current diabetes treatments, although several limitations persist. Microencapsulation devices have been designed for *in vivo* transplantation and retrieval. However, their large geometrical scale hinders adequate mass transfer of nutrients and insulin into and out of the encapsulated device. Microencapsulation technologies have improved their mass transfer properties. However, their complete retrieval remains challenging if the graft becomes a life-threatening problem for the recipient (Ernst et al., 2019).

Several studies have proposed a novel, non-traditional design of cell microcapsules with a toroidal structure (Zhang et al., 2018). A torus is a geometric shape similar to a donut or a ring (Gardner, 1975). These donut-shaped capsules have a large surface area, higher than traditional spherical capsules of the same volume, which improves mass transfer.

In a study conducted by Chen et al. (2019), capsules of different geometries were fabricated, including toroidal, rod, and spheroid shapes, with a similar number of cells per microcapsule using initial cell seeding densities of 3.5, 5.8, and 9.3 million cells/mL, respectively. Results demonstrated that the average number of cells per microcapsule for toroidal, spheroid, and rod-shaped capsules was approximately 12,000. The mean percentage of dead cells was lowest (11%) for toroidal capsules and highest (25%) for spheroids.

Toroidal microcapsules also possess the ability to be interconnected into chains, which theoretically allows for their retrieval after implantation. Additionally, multi-compartmental toroidal capsules have also been developed, which could enable the co-encapsulation of other components (Ernst et al., 2019).

These unusual capsules are formed through vortex rings generated by dropping a droplet into a miscible liquid. Upon impacting the surface of a miscible liquid at a sufficient impact velocity, the droplet begins to deform to dissipate energy by curling backward. As the rim continues to curl, the center of the droplet becomes thinner and thinner. Eventually, when the center is too thin to sustain surface tension, it breaks up, forming a toroidal ring (Agbaglah et al., 2015).

An et al. (2016) presented an efficient method for mass-producing chitosan and alginate toroidal microcapsules using an industrially scalable electrospraying technique (Figures 3 and 4).

Immunomodulators

It is well known that one of the most prominent obstacles to performing

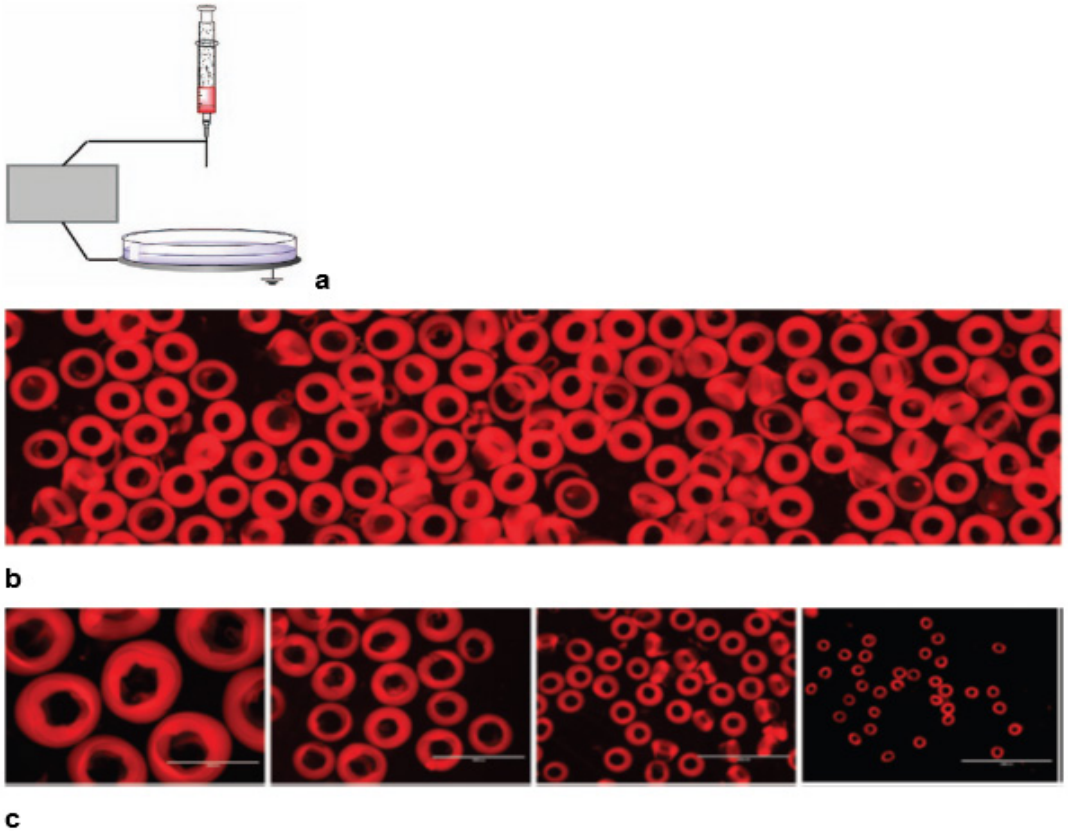

Figure 3. Mass production and size/shape control. (a) Schematic illustration of the electrospray setup. (b) Microscopic fluorescent images of the nanoclay hydrogel donut-microVRP fabricated by electrospraying. For better visualization, a fluorescent dye Rhodamine B was mixed in the nanoclay solution. (c) Size control of the nanoclay hydrogel donut-microVRP. Taken from An et al., 2016

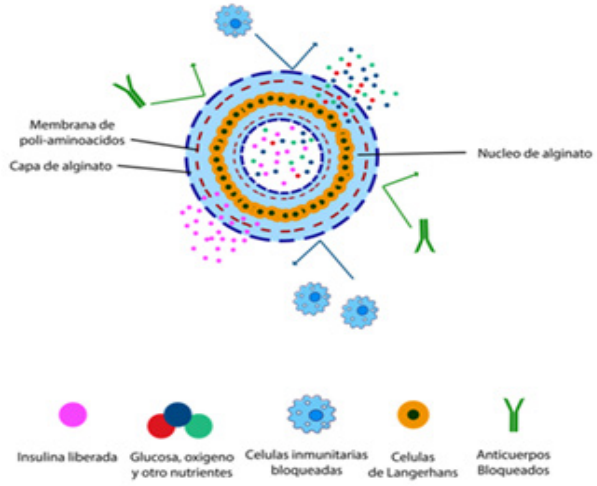

Figure 4. Structure of Toroidal Microcapsules. This is a schematic illustration of the structure of toroidal microcapsules. They possess a donut shape, a larger diffusion surface area, and a shorter diffusion distance, thus allowing for greater viability. They block the entry of immune system cells and antibodies while allowing the entry of glucose, oxygen, and other nutrients and the exit of insulin being released by Langerhans beta cells.

any transplant is the immune reaction to the implanted grafts. Although microencapsulation is a technique that inherently seeks to create a state of immunoisolation, scientific evidence suggests the use of additional techniques to attenuate the Foreign Body Reaction (FBR) and thereby ensure the long-term functionality and viability of the transplanted islets (Mellor and Munn, 2008; Gibly et al., 2017; Calafiore, 2018; Tomei, 2018; Kogawa et al., 2020). Consequently, immunosuppression emerged as an initial alternative to prevent graft rejection; however, given the multiple adverse effects that this measure entails, new strategies focused on modulating the immune system without the need for harmful immunosuppressive agents are currently

under development (Opara, 2017 a,b; Omami et al., 2017).

Among the most relevant techniques for improving islet transplantation outcomes are incorporating immunomodulatory molecules and biomaterials into microcapsules, cell co-transplantation, and antigen-specific immunomodulation (Desai and Shea, 2017; Basta et al., 2021). Experimental studies have incorporated various immunomodulatory molecules into the capsules to stimulate the recruitment of immune suppressor cells or polarize them toward an anti-inflammatory phenotype (Dimitrioglou et al., 2019). Among these are TGF-β1, CXCL12, CCL22, UDCA, HMGB1, MCP1, IL-10, IL-4, IDO1, LTB4, IL-33, and PGE2 (Luo et al., 2008; Mooranian et al., 2017; Alagpulinsa et al., 2019 a,b; Elnashar et al., 2020; Safley et al., 2020). Overall, the results of these studies have been favorable, demonstrating improvements in local inflammatory status and, consequently, in the graft's viability, functionality, and survival (Agesjö et al., 2015; Liu et al., 2016;).

In addition, modulation of specific antigens has been proposed as a potential replacement for conventional systemic immunosuppressive therapies. This strategy consists of decreasing FBR through the induction of T cell anergy, which is another mechanism of lymphocyte tolerance, in which the lymphocyte is functionally unresponsive after antigen encounter but remains alive for extended periods in a hyporesponsive state; expansion of Regulatory T cells (Tregs), which are a specialized subpopulation of T cells that act to suppress immune response, thereby maintaining homeostasis and self-tolerance; or deletion of reactive T lymphocytes in regions surrounding the transplant exclusively (Pearson et al., 2017; Serra and Santamaria, 2019; Clough et al., 2020; Basta et al., 2021).Finally, while this section focuses on immunomodulators, other elements capable of decreasing the severity of FBR without interfering with immunomodulation are worth mentioning. These include conformal coatings, multilamellar capsules, anti-biofouling molecules, and polymer brushes, as well as the proper selection of polymers for microcapsule manufacturing and their purification level (Hwang et al., 2017; Bochenek et al., 2018; Ramezanzadeh Andevari et al., 2018; Calafiore, 2018; Toda et al., 2019; Hu and de Vos 2019; Watanabe et al., 2020; Gattás-Asfura et al., 2020).

Additionally, cell co-encapsulation technologies have recently been implemented, where pancreatic β-cells are encapsulated together with mesenchymal cells (MSCs) (White et al., 2009; Ben Nasr et al., 2015). This approach takes full advantage of the microencapsulation's immunoisolating properties, protecting both pancreatic and MSCs from the host's immune system. This increases their half-life without affecting their secretory function of insulin, cytokines, and angiogenic (SCF, TPO, CXCL12) and immunomodulatory proteins attributed to these cells (Laporte et al., 2019; Kim et al., 2019; Laporte et al., 2020). Indeed, several studies have demonstrated the positive influence of MSCs on cell survival and local inflammatory state reduction, presumably through increased expression of immunomodulatory genes related to IL-6, IL-10, CCL2, CCL5, TGF-β, NOS-2, TSG-6, mPGES-2, and COX-2 (Mao et al., 2019).

Oxygen Enhancers

Another major obstacle to overcome in microencapsulated beta cell transplantation

is the hypoxic conditions to which the graft is subjected after implantation. Disruption of oxygen and nutrient supply is a common phenomenon, especially during the first week after transplantation, due to factors such as abrupt rupture of adjacent capillaries during inoculation and defects in blood flow distribution that particularly affect the central cells of the encapsulated islets (White et al., 2020).

Since hypoxic conditions can themselves determine the success or rejection of the procedure, different strategies have been developed around the local revascularization of the graft, as well as the direct supply of oxygen to the interior of the microcapsules (Groot Nibbelink et al., 2018). One of the most popular strategies in this field is the use of revascularization-promoting biomaterials or pre-vascularized devices. An example of this is the experimental study by Weaver et al. (2018), where a specialized device with a hydrogel center linked to a polyethylene dithiol (PEG dithiol) and an external vasculogenic layer with proteolytic peptides was used as a transport medium for encapsulated islets. This study demonstrated increases in local vascularization and cell viability after implantation. Similarly, other pre-vascularized devices synthesized with biopolymers such as alginate, collagen type I (COL1) or polycaprolactone have incorporated additional techniques such as the addition of gels enriched with growth factors, surface patterns to increase the adhesion of potentially angiogenic cells, porous membranes to facilitate nutrient exchange or the stimulation of fibrinogen, thus generating increases in the survival, functionality and viability of the graft by mitigating local hypoxia (Marchioli et al., 2015; Zbinden et al., 2021; Mridha et al., 2022).

Likewise, techniques such as co-encapsulating pancreatic islets have recently gained relevance. In this context, pancreatic beta cells are microencapsulated together with potentially angiogenic cells that facilitate revascularization, such as endothelial cells, fibroblasts, and particularly pluripotent mesenchymal cells, through the stimulation of factors such as vascular endothelial growth factor (VEGF), kinase insert domain receptor (KDR) or hepatocyte growth factor (HGF) (Cavallari et al., 2012; Zhang et al., 2013; He, 2017; Groot Nibbelink et al., 2018; Kogawa et al., 2020; Nilsson et al., 2020). Other compounds, such as bilirubin, have exhibited beneficial antioxidant effects for the survival of pancreatic β-cells under hypoxic conditions. This molecule has been shown to interfere with hypoxia-inducing apoptotic pathways through the downregulation of TNF-α and the upregulation of anti-apoptotic genes such as haeme oxygenase (HO-1) and anti-apoptotic B-cell lymphoma-2 (BCL-2) (White et al., 2020). These findings were demonstrated in the study where nanoencapsulation of bilirubin in chitosan-127-pluronic particles led to increases in cell viability, as well as reductions in hypoxia and apoptosis levels (Fullagar et al., 2017). Finally, specialized devices have been designed to meet the oxygen demands of transplanted cells. In this regard, the bioartificial device called β-Air consists of pancreatic islets suspended in an alginate hydrogel and a gas chamber that houses oxygen, both covered by an internal membrane permeable to gaseous substances, as well as an external membrane and a mechanical support mechanism (Barkai et al., 2013). In addition, encapsulation of pancreatic islets using the TheraCyte™ device has been shown to protect against allograft

rejection in nonimmunized recipients. The TheraCyte™ system for encapsulating and transplanting cells is a thin membrane-based polymeric chamber. It is fabricated from biocompatible membranes that protect allogeneic cells from rejection by the recipient and, when implanted subcutaneously, induce the development of blood capillaries close to the membranes. This device also has a series of membranes that aim to achieve immuno-isolation of the graft and simultaneously promote local vascularization thanks to an angiogenic outer layer (Kumagai-Braesch et al., 2013).

Conclusions

Pancreatic islet transplantation has emerged as a solution to the limitations of conventional procedures. It significantly improves the quality of life for patients by freeing them from lifelong dependence on exogenous insulin administration and reducing the associated adverse effects. Despite the challenges inherent in transplantation, such as autoimmune reactions, islet microencapsulation is a promising technique to ensure transplanted cells' viability and successful function.

While persistent hurdles exist regarding graft compatibility, nutrient diffusion, and local immunomodulation, pancreatic islet transplantation using cellular microencapsulation techniques has shown successful outcomes. Nevertheless, further long-term clinical research is required to offer accurate and representative assessments of this procedure's efficacy. Additionally, it is essential to continue implementing novel strategies to optimize microencapsulation devices and overcome the existing challenges.

The unexplored combined use of some of the most innovative strategies, technologies, and techniques could potentially solve the current problems that prevent the commercialization and global use of β-cell microencapsulation.

Firstly, achieving effective immunoisolation using biopolymer capsules would open the possibility of using cells of non-human origin. This strategy would address the challenge of biocompatibility by isolating insulin-producing cells from the patient's immune system, thus preventing rejection. In addition, by complementing this technique with the co-encapsulation of MSCs, which possess immunomodulatory properties, not only would the lifespan of the encapsulated cells be extended, but local angiogenesis at the implantation site would also be stimulated.

Furthermore, the use of toroidal capsules, which, due to their geometrical properties, increase the surface area for contact, facilitating oxygen transport to most of the cellular content, and which are manufactured with hybrid biopolymers or with PEOT PBT300, would ensure proper immunoisolation, generating minimal or no inflammatory response, while also preserving essential structural characteristics such as elasticity and resistance to breakage. Thus, by combining these advances, it could be possible to reach the ideal formula to overcome most of the current limitations of cell encapsulation and treat or even cure DM in the future.

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