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MODERN PANCREATIC ISLET ENCAPSULATION TECHNOLOGIES FOR THE TREATMENT OF TYPE 1 DIABETES

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The review includes the results of analytical research on the problem of application of pancreatic islet encapsulation technologies for compensation of type 1 diabetes. We present a review of modern encapsulation technologies, approaches to encapsulation strategies, insulin replacement technologies: auto-, allo- and xenotransplantation; prospects for cell therapy for insulin-dependent conditions; modern approaches to β-cell encapsulation, possibilities of optimization of encapsulation biomaterials to increase survival of transplanted cells and reduce adverse consequences for the recipient. The main problems that need to be solved for effective transplantation of encapsulated islets of Langerhans are identified and the main strategies for translating the islet encapsulation technology into medical reality are outlined.

Keywords: pancreas, islets of Langerhans, encapsulation, transplantation, immunosuppression, type 1 diabetes.

INTRODUCTION

Type 1 diabetes mellitus (T1DM) is a multifactorial disease characterised by a relative or absolute deficiency of insulin secretion, leading to chronic hyperglycemia and other metabolic disorders. Diabetes has been shown to develop with over 90% decrease in pancreatic islets, and for a patient with an average body weight, 300,000 viable active islets are enough to control blood sugar [1].

A promising option for the treatment of insulin-dependent carbohydrate metabolic disorders is the use of transplantation of insulin-producing beta cells as part of the islets of Langerhans or a whole organ into the recipient to activate the biological feedback mechanisms of glycemic feedback and insulin production [2].

To reduce the burden of autoimmune disease and increase cell survival, various approaches have been proposed: from the use of steroid-free immunosuppression schemes [3] to transplantation of induced pluripotent stem cells (iPSCs) and mesenchymal stem cells (MSCs), committing to beta cells [4], and the use of immune-independent insulin-producing organoids [5].

Recently, the most promising solution to the problem of immunosuppression is considered encapsulation of transplantable pancreatic islets, also known as islets of Langerhans (IL) to protect them from immunocompetent cells.

ISLET ENCAPSULATION

This paper reviews the main strategies and ways of solving the problems of effective functioning of transplanted IL as part of micro- and macro-units in insulin-dependent disorders in the recipient's body. On the way to the goal set, a whole set of multi-component and interdependent problems, from the chemical structure of the capsule wall to determining the optimal location for transplantation of encapsulated IL, will have to be solved (Fig. 1).

MATERIALS AND CAPSULE DERIVATION

The ideal polymeric capsule for IL encapsulation, according to the literature [6], should meet at least the following criteria:

– let insulin into the blood, and oxygen, glucose, etc. into the cells;
– do not let white blood cells, phagocytes through;
– be compatible with both the encapsulated cells and the recipient's body, so as not to cause immunological and fibrotic reactions;
– have a smooth topography without a rough surface;
– stimulate vascular growth around the capsule (for better supply of the encapsulated cells with nutrition and rapid "drainage" of the released insulin).

In the vast majority of cases, capsules made of hydrogel-forming natural and synthetic polymers have these characteristics [7].

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Natural polymers

The most commonly used natural polymers for creating IL microcapsules are agarose collagen, chitosan, alginate, cellulose, their mixtures and numerous chemical modifications.

It has been found that the immunoprotective properties of agarose gels [8–9] can be controlled by changing the agarose concentration during gel formation. Typically, 5% agarose is used to create capsules, but by increasing the agarose concentration from 5% to 7.5–10% or by applying other polymers to the capsule surface, the graft survival time in vivo can be increased [11]. To this end, Dupuy et al. [12] coated agarose microcapsules with polyacrylamide; another successful approach was to coat the agarose surface with polybrene and carboxymethyl cellulose (CMC) [13]. To create these capsules, complex mixtures consisting of 5% agarose and 5% polystyrene sulfonic acid incubated with polybrene and CMC were formed.

To stimulate cell growth in the graft system, agarose can be supplemented with other polymers: for example, collagen-agarose macrogranules showed a better effect on rat IL functionality compared to those containing agarose alone. IL encapsulated in these macrogranules were able to maintain normoglycemia for up to 170 days in diabetic mice in a streptozotocin-induced diabetes model [14].

Despite the many studies conducted using agarose and its derivatives, two major drawbacks of agarose capsules for IL confinement can be noted:

1) large scatter of obtained gel balls, 100 to 1000 μm in size. This is related to the capsule derivation method – mainly temperature-induced suspension gelation methods are used.
2) the presence of toxic molecules in the agarose itself due to insufficient purification of natural materials [15].

Alginate is an anionic polysaccharide derived from different species of algae, which significantly affects the physical and chemical properties of alginate microcapsules [16].

To reduce permeability and increase the stability of alginate capsules, the polycationic layer is usually added to the core of the alginate gel as a second layer, followed by an outer layer of alginate. The most commonly used polycation is poly-L-lysine, although other polycations such as poly-L-ornithine can also be used. For example, microcapsules containing alginate-poly-L-ornithine instead of alginate-poly-L-lysine-alginate (APA) provided better graft survival with porcine IL when xenotransplanted to Cynomolgus monkeys [17, 18].

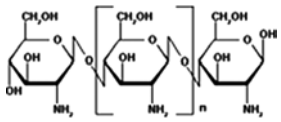
Organization levels	Research targets	Tasks to be solved
	Capsule materials and production	Biopolymers, synthetic polymers, encapsulation with bioactive molecules, capsule formation methods
	Encapsulation strategy (physical parameters of capsules)	Capsule size, permeability (pore size), capsule wall thickness and elasticity
	IL cells in in capsules	Allogeneic, xenogeneic, satellite (supporting) cells, alternative sources of β-cells
	Physiological parameters of capsule environment	Oxygenation, vascularization, fibrosis
	Site of graft insertion into the recipient's body	Abdominal cavity (omental bursa), renal capsule, subcutaneous space

Fig. 1. Multifunctional tasks in the transplantation of encapsulated IL

However, APA microcapsules suffer from a significant drawback: the polycationic coating (PCC) degrades over time and is considered highly immunogenic, making APA capsules unstable in the long term. It has been demonstrated that cross-linking high α-L-guluronic acid alginate with Ba^{2+} ions results in capsules with less permeability to IgG and greater biocompatibility than when cross-linked with Ca^{2+} ions [19].

Animal studies [20, 21] have demonstrated the ability of barium alginate microcapsules to provide long-term immune protection in both allo- and xenotransplantation. However, even in the absence of immunogenic PCC, transplantation of barium alginate microcapsules led to pericapsular fibrotic overgrowth (PFO) [21]. Barium alginate microcapsules and purified alginate [22] do not cause PFO when tested in small animals, such as rodents, but cause severe PFO when transplanted into a large animal such as a baboon.

Chitosan, a basic cationic polysaccharide derived from chitin [23], has not been tested as extensively as alginate or agarose for immunoprotection studies, since chitosan in its salt-free form is insoluble in aqueous solutions except for low molecular weight samples. At the same time, it can be used as an additive in the matrix. It is suggested that the use of chitosan instead of poly-L-lysine can provide higher mechanical strength and stability due to the strong bond between chitosan and the alginate gel [24].

Collagen, a fibrillar protein, is considered one of the most versatile polymers for encapsulating various cell types. To date, 29 types of collagens have been identified and described [25], but nevertheless, type I collagen accounts for 90% of the total and is the most frequently used polymer for encapsulation [26]. Collagen capsules need to form a complex with other polymers or a protective layer for long-term use in biomedical applications [27, 28].

Glutaraldehyde is the most widely used crosslinking agent, including for collagen in model cell systems, but it causes an inflammatory response in the recipient's body [29].

Synthetic polymers

Despite the stability of characteristic properties of synthetic polymers [30], cell encapsulation procedures require the use of toxic solvents [30, 31], which negatively affects biocompatibility of capsules.

Polyethylene glycol (PEG) is most frequently used for cell encapsulation. It is acceptable for encapsulation of a wide range of cells: IL [32], chondrocytes [33], osteoblasts [34], MSCs [35]. PEG is obtained by polymerization of ethylene glycol oligomers in the presence of acid or alkaline catalysts. But when PEG monomers are terminated with methacrylate or acrylate groups, they can undergo rapid crosslinking when exposed to ultraviolet or visible light in the presence of appropriate photoinitiators. Photoinitiators create free radicals that can initiate the formation of photopolymerizable hydrogels [36].

Over the past two decades, many different IL encapsulation procedures using PEG have been applied, but photopolymerization of PEG-diacrylate polymers and gelation based on a combination of physical and chemical cross-linking have become the main methods [37]. Nevertheless, many studies describe the emergence of an immune response to PEG-encapsulated cells. For example, J.Y. Jang et al. noted that PEG grafted onto the collagen capsule can inhibit lymphocyte activation but not macrophages [38]. As an enhancement of immunoprotection, groups of researchers suggest modifying the exosurfaces of PEG capsules with immune cell receptor's such as Fas ligand (FasL) [39] and tumor necrosis factor receptor 1 (TNFR1) [40].

ENCAPSULATION STRATEGIES

IL encapsulation strategies can be divided into three main categories: macroencapsulation, microencapsulation and nanoencapsulation (Fig. 2). The first two are recognized as the most promising.

Macroencapsulation is the encapsulation of several thousand ILs in a macroencapsulation device more than 1000 μm in diameter. Depending on the transplantation site, macroencapsulation devices can be divided into extravascular and intravascular.

Intravascular macroencapsulation usually involves the placement of multiple ILs in hollow semipermeable fibers, which are then directly connected to the host vasculature through anastomoses. Despite promising studies using intravascular devices, researchers have reported severe problems with embolization and blood clot formation. This has prevented the Food and Drug Administration (FDA) from approving these systems for clinical trials [42].

Extravascular macroencapsulation usually involves placing multiple ILs in simple diffusion chambers that do not require intravascular shunts. Such devices are often placed in the abdominal cavity or under the skin, from where they can be retrieved and repaired in case of damage.

Extravascular macrodevices are in the form of tubular or flat diffusion chambers. The tubular device is structurally weak and can break, and requires a large amount of IL for seeding [43]. Flat devices are structurally more stable. For example, the Islet Sheet device from Islet Sheet Medical (USA) has been shown to provide good graft survival in both allogeneic and xenogeneic transplantation [44, 45]. The main disadvantage of the Islet Sheet device is the limited oxygen diffusion leading to hypoxia and necrosis of the central groups, implanted IL.

The problem of limited oxygen diffusion is tackled by several approaches to the design of macrodevices. For example, the TheraCyte™ macroencapsulation device is

equipped with an external membrane that promotes neovascularization [46]. IL encapsulated in TheraCyte™ devices survived for a long period of time in both allo- and xenotransplantation models [47]. A modified version of TheraCyte™ device, namely Encaptra® system (EN250 device), developed by ViaCyte (USA), is currently being tested for safety in a phase II clinical trial [48].

The problem of hypoxia can also be addressed by means of an artificially oxygenated β-Air Bio-artificial Pancreas (BAP) device developed by Beta-O2 Technologies Ltd (Israel) [49]. This device consists of a semi-permeable chamber containing IL immersed in alginate hydrogel, and an additional compartment that provides daily oxygen supply through an external probe system [50]. Preliminary studies with small-sized BAP devices implanted in diabetic pigs showed that the encapsulated allogeneic IL preserved its function and blood glucose levels dropped to normal for several months [51].

In in vitro experiments, perfluorocarbons and calcium peroxide (CaO_2) were added to IL-containing hydrogels to increase the rate of O_2 diffusion in the hydrogel system [52]. This could also be a promising solution for strategies to overcome IL hypoxia in macroencapsulation devices.

Microencapsulation is the incorporation of one or more IL into microcapsules ranging from 200 to 1500 μm in size (Fig. 3).

This technology has several advantages over macroencapsulation. First, the microcapsules are generally spherical in shape, thereby providing a greater surface area to volume ratio and increased transport of oxygen and nutrients required for IL survival. Secondly, microcapsules are mechanically stable and easier to manufacture, giving the freedom to change parameters such as capsule size, permeability and thickness. Thirdly, they can be implanted using a minimally invasive procedure, and the smooth spherical geometry minimizes the immune response to the foreign body. The main disadvantage is the difficulty of extracting microcapsules from the transplant site.

The need to ensure maximum cell survival and preserve their normal viability impose the following restrictions on the conditions of the IL microencapsulation procedure:

– exclusion of organic solvents;
– carrying out the procedure in an aqueous solution isotonic to the cell cytosol (in a phosphate-buffered saline);

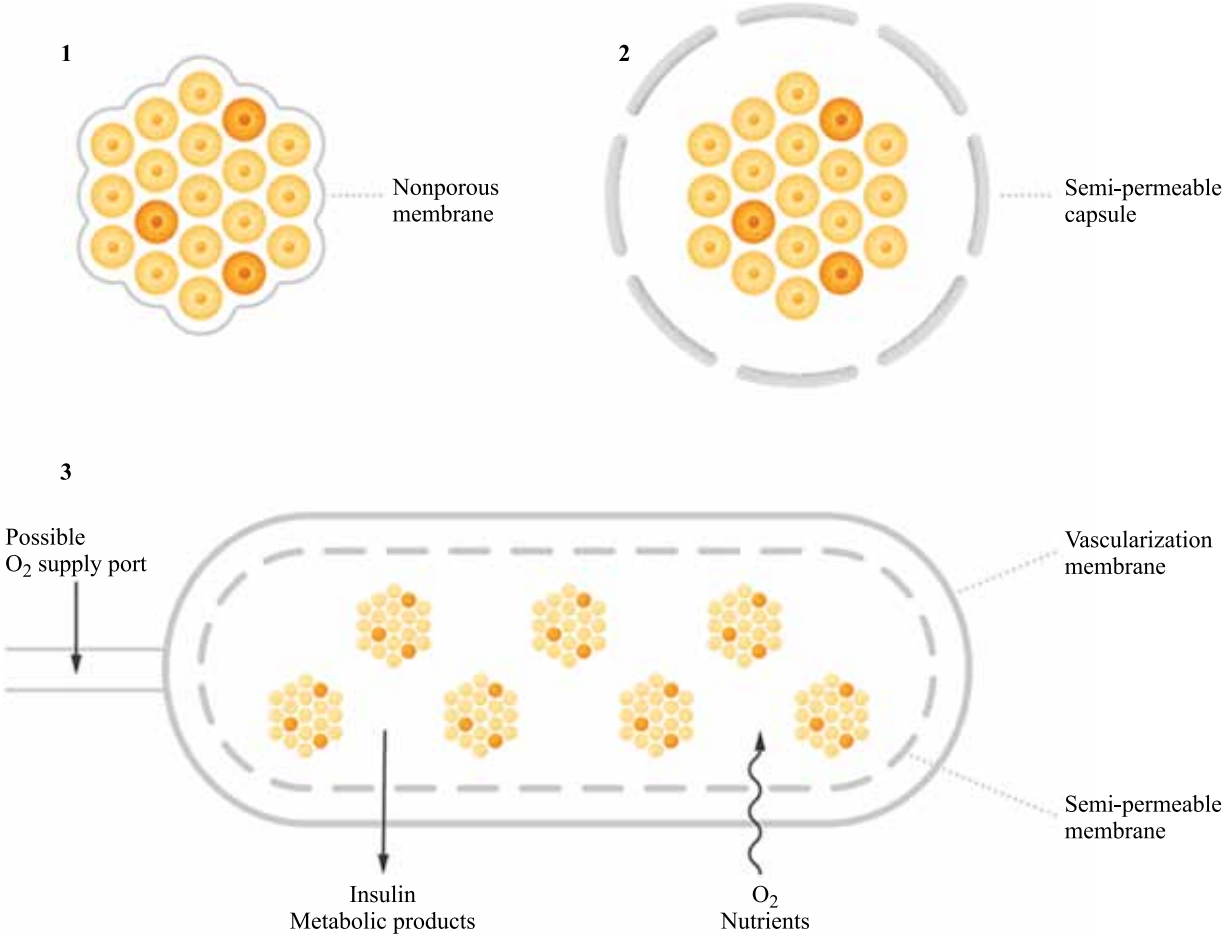


Fig. 2. The main strategies for encapsulation of IL [14]: nanoencapsulation (1), microencapsulation (2), macroencapsulation (3)

– maintaining the pH between 7.2 and 7.5;
– at a temperature between room temperature and 40°C (ideally at 37°C in an atmosphere of 5% carbon dioxide saturated with water vapor);
– the solution should quickly form a gel for even distribution of cells or IL and prevention of sedimentation.

All this significantly narrows the range of materials intended for use, and the above-mentioned conditions correspond well to the polymeric hydrogels discussed above. The most popular natural polymer for IL microencapsulation is sodium alginate, which can form hydrogels quickly in the presence of divalent ions at neutral pH and moderate temperatures [53, 54].

Using sodium alginate as an example, let us consider what the main problems researchers encounter in IL microencapsulation are. The literature identifies several factors that are crucial in the engraftment of microencapsules with IL.

Alginate purity is one of the main factors affecting biocompatibility: alginates obtained from natural sources contain immunogenic contaminants (proteins, polyphenols, endotoxins) [55], which often leads to poor graft survival due to the appearance of PFO [56]. Microcapsules derived from insufficiently purified commercial alginates activate the immune system and induce the release of inflammatory cytokines IL-1β, TNF-α and IL-6 from murine and human monocytes and macrophages [57]. One study aimed to screen for impurities and found that commercial alginate labeled as "ultrapure" still contained impurities such as peptidoglycan and lipoteichoic acid [58]. The same authors proposed the development of a screening assay for identification of pathogen-associated molecular structures in alginate polymers [58].

The composition of alginate also plays an important role in determining biocompatibility, since the G/M ratio strongly influences the physicochemical properties of microcapsules. High-G alginate microcapsules are more stable compared to high-M alginate, whereas high-M alginate microcapsules can provide selective permeability to immunoglobulins and immune cells, thereby providing better immunoprotection [59]. However, some studies have reported that high-M alginate microcapsules are more immunogenic, leading to PFO [60], while other studies have reported the opposite effect [61].

In addition to the G/M ratio, the viscosity and molecular mass (MM) of alginate also play an important role in determining biocompatibility. S. Schneider et al. demonstrated that low-MM alginate microcapsules cause PFO, and stressed the need to remove low molecular mass fractions during the purification procedure to increase biocompatibility [62].

Another important factor is the geometry and size of the capsule. Traditional IL microcapsules are spheres with a fixed diameter of 700–1500 μm. There are several opposing opinions on this issue: small 250–350 μm microcapsules have been shown to be biocompatible and contribute to a smaller PFO compared to traditional ones (500–800 μm) when transplanted into rats [63] and monkeys [64]. On the other hand, O. Veiseh et al. showed that

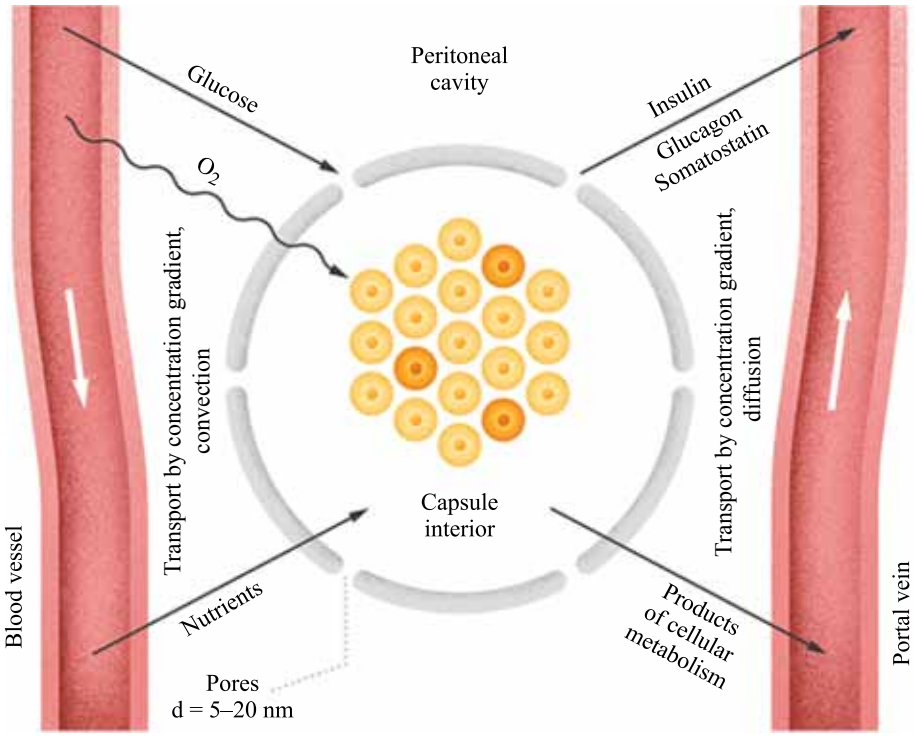


Fig. 3. Model of the structure of microencapsulated IL and its functions

larger 1500 μm alginate microcapsules have better biocompatibility and significantly reduced PFO compared to 500 μm capsules when xenotransplanting both groups into C57BL/6 mice and primates [65]. The authors also demonstrated that ILs encapsulated in larger 1500 μm capsules remained viable, had higher insulin kinetics and provided better glycemic control under xenotransplantation conditions with significantly less PFO compared to smaller microcapsules at 180 days.

Nanoencapsulation is the coating of a single IL with a biopolymer material to form 70–150 μm structures. The most common nanoencapsulation method is layer-by-layer deposition (LbL) of oppositely charged biomaterials on the IL surface (nanoencapsulation).

Various coatings have been developed for LbL with individual physicochemical properties. Haque et al. xenotransplanted primate IL encapsulated by LbL using 3 polymers in immunosuppressed mice. The encapsulation showed uniform nanoscreening of the polymers on IL without loss of cell viability and function [66]. Park et al. transplanted IL with nano-screened heparin to primates, which was shown to reduce instantaneous blood inflammatory reactions using similar nano-screening [67]. Another group used ultra-thin heparin-polymer nanofilm as a platform to incorporate biological mediators to modify the IL surface [68].

PANCREATIC ISLET CELLS IN CAPSULES

In addition to ensuring a high degree of survival of IL cells over a long period of time while maintaining their ability to produce insulin, there is also the problem of shortage of donor healthy, viable beta cells.

Allogeneic IL cells have been successfully used as donor material in the treatment of diabetes since 2000. However, as early as 1994, R. Soon-Shiong et al. [69] conducted the first successful test with microencapsulated IL in alginate-poly-L-lysine: allogeneic IL were transplanted intraperitoneally to a patient with type 1 DM, which reduced blood glucose levels for 9 months. After that, two more groups of researchers, R.C. Calafiore et al. [70] and B.E. Touch et al. [71], made attempts to transplant allogeneic IL microencapsulated in modified alginates, but the fall in glucose levels in both cases was not enough.

Porcine xenogeneic IL cells are a promising source of IL transplantable to humans for any of the following reasons: the similarity of pig and human insulin, high fecundity of pigs, availability of effective and accurate methods of genetic modification of pigs [72].

The most widely used are IL from adult donor females or neonatal islet-like cell clusters (NICC) are most commonly used; experiments with porcine fetal islets, as well as with the buds of the IL from embryos, are known [73].

Currently, there are three main strategies to increase the viability and prolong the functioning of porcine IL in the recipient's body:

1) free porcine IL transplantation according to immunosuppression and tolerance protocols;
2) encapsulation of porcine IL, in which case global immunosuppression is not required;
3) genetic modification of porcine IL and subsequent use with advanced low-toxicity immunosuppression.

However, there are risks of using porcine IL: first of all, porcine endogenous retrovirus (PERV) sequences that can be activated after xenotransplantation [74].

Second, there is a risk of developing a superacute immunological rejection reaction due to human Gal (Galactose-1,3-Galactose) antigens reacting to the porcine cell membrane disaccharide. Binding of antibodies to Gal antigens leads to almost immediate activation of the complement system with subsequent destruction of the graft. Several groups of transgenic pigs have been created to overcome the superacute reaction of immunological rejection:

1) knockout by Gal;
2) with transgenic expression in IL cells of human protein regulating the complement system (hCD46);
3) with transgenic expression of LEA29Y (a high-affinity variant of the T cell co-stimulation inhibitor CTLA-4Ig) under control of porcine insulin gene [75].

It is possible that the dual combination of immunosuppression inhibitors – IL encapsulation from transgenic pigs can provide effective graft protection without the need for strong immunosuppressive agents [76].

Satellite cells are co-cultured in the same macro- or micro-object with IL cells. Sertoli cells have been widely studied as supporting immunomodulatory "companion cells": co-transplantation of unencapsulated IL with Sertoli cells has proven useful for increasing graft survival in allo- [77], xeno- [78] and autotransplantation models [79]. Moreover, co-encapsulation of IL with Sertoli cells secreting immunosuppressive factors improves xenograft survival [80].

The immunomodulatory properties of MSCs are widely known and have been used in several studies to increase IL survival and improve transplant outcomes [81, 82]. In addition, some studies have reported increased insulin secretion as an advantage of co-encapsulating IL with MSCs [83].

Genetically modified cells have also been used to improve IL survival: co-encapsulation of IL with mouse bioengineered Sertoli cells (TM4) producing insulin-like growth factor-II (IGF-II) improves β cell survival and provides better glycemic control [84].

Alternative sources of beta cells. In addition to the above-mentioned sources of donor beta cells, methods for obtaining normally functioning insulin-producing cells from various human cell populations are being actively developed [85] in order to obtain patient-specific cellular products.

Pluripotent stem cells – embryonic stem cells (embryonic SCs or ESCs) and iPSCs – are mainly considered as a product for cell therapy. The use of pancreatic progenitor cells derived from human ESCs to treat patients with type 1 DM is at the experimental stage: the cells are encapsulated in Encaptra® macrosystem [86].

Protocols for iPSCs differentiation with additional steps were developed, optimized by cocktails of inducing factors and chemicals, using 3D cultivation methods, which allowed to obtain cell clusters morphologically and functionally similar to pancreatic islet cells [87].

Mesenchymal stem cells. The use of MSCs in diabetes is possible in two ways: differentiation into insulin-producing cells [88] and direct injection of undifferentiated MSCs [139]. When cultured in media containing fibroblast growth factor, adipose tissue-derived MSCs can express the Isl1 marker, which is necessary for islet cell formation [89]. Human umbilical cord blood-derived MSCs contain the genes required for differentiation into endocrine prostate tissue (Isl1, PDX1, Pax4 and Ngn3) [90], so they release insulin and C-peptide in response to glucose stimulation in vitro and in vivo.

Direct reprogramming for beta cells implies the use of DNA integration (using viral vectors in most cases) into cells of different types, which leads to creation of beta cells, bypassing their return to the pluripotent state. As a starting material for direct reprogramming, pancreatic ductal cells, acinar tissue, alpha cells and others are used.

It has been shown that a combination of three beta cell regulators – NGN3, PDX1 and MAFA – can effectively transform pancreatic adult mouse acinar cells into beta-like cells using an adenoviral vector [91].

Studies have shown that gastrointestinal epithelial cells can also be transformed into beta-like cells. Gastric antrum cells seem to be particularly susceptible to such transformation. In a separate study, conditional removal of Foxo1 from Ngn3+ intestinal endocrine progenitor cells led to formation of insulin-producing cells in the intestine [92].

Other examples of murine cell reprogramming include: cytokine-mediated conversion of acinar cells to insulin-expressing cells, conversion of ductal cells to insulin-expressing cells by FBW7 deletion and conversion of hepatocytes to insulin-producing cells using TGIF2 [93]. Extreme loss of β-cells can spontaneously transform δ- and α-cells of the pancreas into β-cells [94].

PHYSIOLOGICAL PARAMETERS OF THE CAPSULE ENVIRONMENT

There is no formed capillary network in the artificially created system of encapsulating devices, so solving the problem of stable trophism and oxygenation of transplanted cells is essential for their survival. A. Pileggi et al. initiated pre vascularization by simulating the physiological reaction of the body to a foreign body: the catheter was injected subcutaneously and removed after 4 weeks [95]. Pre-vascularization of the transplant site can also be achieved by pre-treating the transplant site with angiogenic factors [96].

Another approach is to embed angiogenic factors into the structure of cell capsules: for example, vascular endothelial growth factor (VEGF) into PEG [97]. G. Marchioli et al. constructed microcapsules from heparinized polycaprolactone, and also observed an increase in angiogenesis in the graft area [98].

Encapsulation of satellite cells together with IL can also lead to increased capsule vascularization. For example, the use of adipose tissue-derived or bone marrow-derived MSCs [99].

In addition, several attempts have been made to improve oxygenation at the capsule transplant site, including generating oxygen near the microcapsules using photosynthesis [100] or an electrochemical generator [101]. Unfortunately, these oxygen generating systems cannot produce enough oxygen required under a clinical setting.

One of the body's defense mechanisms against intrusion is fibrous overgrowth around the foreign object [102]. In microcapsules, the reduced diameter and greater surface area to volume ratio contribute to improved diffusion, which is indirectly confirmed by faster response of microencapsulated islets to changes in glucose in the bloodstream [103].

TRANSPLANTATION SITE IN THE RECIPIENT'S BODY

The ideal site for transplantation should have such features as low immune exposure, ease of extraction of implanted capsules, access to the recipient's vascular network with the possibility of neovascularization of the implanted graft and sufficient space to accommodate the desired number of implanted microcapsules [104]. Non-encapsulated ILs are usually injected into the liver through the portal vein. Portal infusion of microencapsulated IL is not possible due to their size. Microencapsulated ILs are usually injected into the abdominal cavity.

However, there are also negative influences when microcapsules are transplanted into the abdominal cavity: insufficient revascularization, high immunogenicity, chronic hypoxic stress, which makes it necessary to have more encapsulated IL to normalize the glucose levels compared to unencapsulated IL [105]. The advantages of transplantation of encapsulated IL into the surgically created omentum in diabetic rodent models have been shown to result in long-term normoglycemia [106]. Another promising IL transplantation site is the kidney capsule. Studies on large animals showed that in two out of seven Cynomolgus monkeys, C-peptide was detected in the blood 60 days after transplantation of microencapsulated porcine IL under the kidney capsule

[107]. However, the renal capsule is highly vascularized, and the possible space to be used limits the introduction of a large graft volume.

The subcutaneous space is another alternative site that is widely used for transplantation of encapsulated IL.

Studies have shown that xenotransplantation of encapsulated IL into the abdominal cavity of C57BL/6 mice results in strong PFO three weeks after transplantation. However, when the same encapsulated IL were transplanted subcutaneously, PFO was significantly reduced [108]. Thus, subcutaneous transplantation of microencapsulated IL can be adopted as a strategy to reduce PFO and increase IL survival, if the problem of poor oxygen supply proves to be solvable.

GLOBAL CLINICAL TRIALS USING ENCAPSULATED IL

A relatively small number of encapsulation systems have been used in clinical trials. Although all of the systems have been shown to be safe for patients, their efficacy has varied [109–116].

In the creation of IL and β-cell encapsulation systems, researchers use different approaches: micro- and macro devices, transplantation sites and methods, allo- and xenografts.

Initially, clinical trials focused on allogeneic IL, but the selective immune barrier of microcapsules allows the safe use of porcine IL as an alternative cell source as well. Living Cell Technologies (LCT) conducted a large clinical trial using porcine IL encapsulated in alginate-poly-L-ornithine called Diabecell®. Eight patients received different doses of IL (from 5000 to 10,000 IEQ per kg of body weight), and six of them showed reduced exogenous insulin levels for up to eight months [117].

As mentioned earlier, macroencapsulation devices limit oxygen transfusion to cells to a greater extent. Therefore, one of the modifications – the BAP device – is designed to solve this problem by using a built-in reusable oxygen cylinder. A phase I study evaluated the safety and efficacy of implantation of a βAir device containing human allogeneic pancreatic islet in patients with type 1 diabetes mellitus. Four patients were transplanted with 1–2 BAP devices, each containing 1,800–4,600 islet equivalents per kg of body weight and were monitored for 3–6 months with regular oxygen restoration. Although β cells survived in the device, only minute levels of circulating C-peptide were observed without any effect on metabolic control. PFO was observed in the capsule environment, and the recovered devices showed a blunted insulin response and amyloid formation in the endocrine tissue [118].

ViaCyte has developed the Encaptra® macroencapsulation system, which, unlike all competitors, incorporates iPSCs rather than IL. ViaCyte is currently conducting a Phase I/II multicenter clinical trial using macroencapsulation technology and the VC-01™ cell product to evaluate the safety and effectiveness of the system over a 2-year period [114].

Unlike Encaptra®, the Sernova Cell Pouch is not immune-isolating. The specificity of its transplantation is aimed at preliminary vascularization of the subcutaneous area before the introduction of cells through the canal. The canal-forming device is inserted under the skin for 30 days to allow vascular integration with the device. The row of rods is then removed to fill the formed channels with encapsulated IL. It is assumed that such stimulation of the microvasculature can significantly increase the survival rate of encapsulated islets by increasing trophism and gas exchange. However, a 3-year phase I/II clinical trial using this device was discontinued in 2016 after recruiting three patients [110].

Encapsulation of pancreatic islets in thrombin-plasma gel is somewhat different from the encapsulation standards: allogeneic IL is resuspended in autologous plasma and laparoscopically distributed over the omentum surface: it has a dense vascularized surface and is easily accessible. In addition, recombinant clinical-grade human thrombin is used for cell adhesion. This method has been used in one patient with restoration of euglycemia and subsequent insulin independence for 12 months [119].

CONCLUSION

New developments in the field of bioactive IL encapsulation, which will make it possible to avoid transplant immunosuppression and achieve long-term functional activity of islet cells, are now extremely necessary in Russia and the global community. Beta cell and IL encapsulation technologies, including nano-, micro-, and macroencapsulation, are promising strategies to the treatment of type 1 diabetes, as they provide transplantation of cell resources without immunosuppressive agents and allow the use of alternative donor sources.

The main problems that need to be addressed for effective transplantation of encapsulated pancreatic islets are related to graft oxygenation, inflammatory response, biocompatibility of the material, and location and method of optimal transplantation. The long-term success of encapsulation strategies can be hampered by pericapsular fibrotic overgrowth and the limited survival of encapsulated islets, especially after intraperitoneal implantation. In each area of encapsulation, there are still limitations that hinder their wide clinical application: macroencapsulation devices are easily retrievable, but contribute more to pericapsular fibrotic overgrowth and less to normal oxygenation and cell trophism. Micro- and nano-capsules are more difficult to retrieve from the recipient's body, but the cells are in them in more satisfactory conditions.

In addition to the above, there is also the problem of shortage of healthy, viable donor beta cells. Porcine IL xenotransplantation is currently the most advanced

alternative to IL transplantation or allotransplantation in the world, especially since recent advances in genetic engineering have led to a reconsideration of the use of porcine organs. Using the clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated (Cas) gene technology [120], a pool of 62 known porcine retroviruses can be removed from pig skin cells, which in principle can also be used to obtain iPSCs, and then genetically "pure" pigs can be used as islet cell donors [121]. It should be noted that xenotransplantation is prohibited in Russia.

Overall, advances in biomaterial science, fabrication techniques, safer implantation strategies, angiogenesis stimulation and cell biology, and new alternative sources of pancreatic islets may make beta cell encapsulation technologies become a medical reality.

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The authors declare no conflict of interest.

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