Islet Encapsulation: Physiological Possibilities and Limitations

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A logical cure for type 1 diabetes (T1D) involves replacing the lost insulin-producing cells with new ones, preferably cells from a well-characterized and unlimited source of human insulin-producing cells. This straightforward and simple solution to provide a cure for T1D is immensely attractive but entails at least two inherent and thus far unresolved hurdles: *1*) provision of an unlimited source of functional human insulin-producing cells and *2*) prevention of rejection without the side effects of systemic immunosuppression. Generation of transplantable insulin-producing cells from human embryonic stem cells or induced pluripotent stem cells is at present close to reality, and we are currently awaiting the first clinical studies. Focus is now directed to foster development of novel means to control the immune system to enable large-scale clinical application. Encapsulation introduces a physical barrier that prevents access of immune cells to the transplanted cells but also hinders blood vessel ingrowth. Therefore, oxygen, nutrient, and hormonal passage over the encapsulation membrane is solely dependent on diffusion over the immune barrier, contributing to delays in glucose sensing and insulin secretion kinetics. This Perspective focuses on the physiological possibilities and limitations of an encapsulation strategy to establish near-normoglycemia in subjects with T1D, assuming that glucose-responsive insulin-producing cells are available for transplantation.

During the past decade, huge efforts have been directed toward the generation of transplantable insulin-producing cells from various stem cell sources, and recently a few reports have been published demonstrating reversal of hyperglycemia in streptozotocin-induced diabetic mice after transplantation (1,2). However, careful scrutiny of the results that have been presented suggests that the field must await further scientific achievements before truly glucose-responsive insulin-producing cells will be available for clinical testing. The particular problems of generating glucose-responsive insulin-producing cells were recently elegantly reviewed and will not further be discussed in this Perspective (3).

The current indications for clinical islet transplantation are *1*) severe unawareness of hypoglycemia and *2*) transplantation to subjects already on systemic immunosuppression, most often because of a previous or simultaneous kidney transplant. At present, there is no major shortage of primary human islets, i.e., those obtained from organ donors, to fulfill the current indications for clinical islet transplantation; a shortage of insulin-producing cells will only occur if we can control immune-mediated rejection of the transplanted cells without the need for systemic immunosuppression. The focus is now on physically protecting the transplanted cells from the immune system by encapsulation (4), and large funding agencies such as JDRF have defined encapsulation as a prioritized area of research (http://www.jdrf.org/research/). The first publications on islet encapsulation appeared about 35 years ago, and, in principle, investigators are still working with the same materials and technical solutions, as recently reviewed in detail (4). The host immune system recognizes a transplanted cell either via direct or indirect antigen presentation. In allogeneic transplantation, the direct antigen presentation dominates and encapsulation prevents rejection by blocking cell-to-cell contact between recipient T-cell receptors and MHC on the transplanted cell. The indirect pathway of antigen presentation constitutes the dominant mechanism of immune activation in discordant pig-to-nonhuman primate islet xenotransplantation (5) but can occasionally also be observed after allotransplantation (6). Proteins released by encapsulated xenogeneic cells are presented in conjunction with recipient MHC on antigen-presenting cells triggering a delayed-type hypersensitivity–like reaction dominated by activated macrophages accumulating around

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the capsule and destroying the grafted cells by soluble toxic compounds and by diminishing transport of oxygen and nutrients over the membrane (7). Inherent with the concept of encapsulation is the ability of almost free passage of insulin and other proteins of similar size emanating from the transplanted islets over the capsule wall. Therefore, from a theoretical point of view, it is almost impossible to avoid the problem with indirect antigen presentation most likely limiting the application of encapsulation for islet xenotransplantation.

This Perspective will focus on the physiological possibilities and limitations of an encapsulation strategy to establish near-normoglycemia in subjects with type 1 diabetes (T1D), assuming that allogeneic glucose-responsive insulin-producing cells are available for transplantation.

GLUCOSE METABOLISM IN SUBJECTS WITHOUT DIABETES

Glucose metabolism in subjects without diabetes is critically dependent on incretins released from the gastrointestinal (GI) tract (8). After a meal, the nervous and GI systems work in concert to potentiate the release of insulin from the native islets in the pancreas. This release already occurs before a significant rise in blood glucose occurs (Fig. 1*A* and *B*) (9,10). After a standardized meal (SM), blood glucose levels rise only slowly during the first 45 min (blue line in Fig. 1*A*), whereas insulin secretion is markedly increased (blue line in Fig. 1*B*). In fact, a rise of only 1–2 mmol/L in blood glucose after a meal results in a release of insulin similar to that induced after intravenous injection of glucose (intravenous glucose tolerance test [IVGTT]) (Fig. 1*C* and *D*) (10). Physiologically even more important is the massive release of insulin (green and black lines in Fig. 1*B*) that occurs about 45–60 min after the intake of an SM, preventing blood glucose from increasing above 7–8 mmol/L (green and black lines in Fig. 1*A*) (10). Furthermore, insulin and glucagon are released from the pancreas in a pulsatile manner, with clear cycles of about 7–8 min. Insulin and glucagon release are shifted in time so that whenever insulin levels peak, glucagon levels are reduced, and vice versa (11,12). This synchronization of insulin and glucagon secretion is thought to be important for optimal physiological effects of both insulin and glucagon on the liver, the critical organ for achieving normal glucose metabolism, which uses about 70% of the hormones released from the pancreas during the first passage.

These intricate and well-coordinated systems enable strict and tight blood glucose control in subjects without

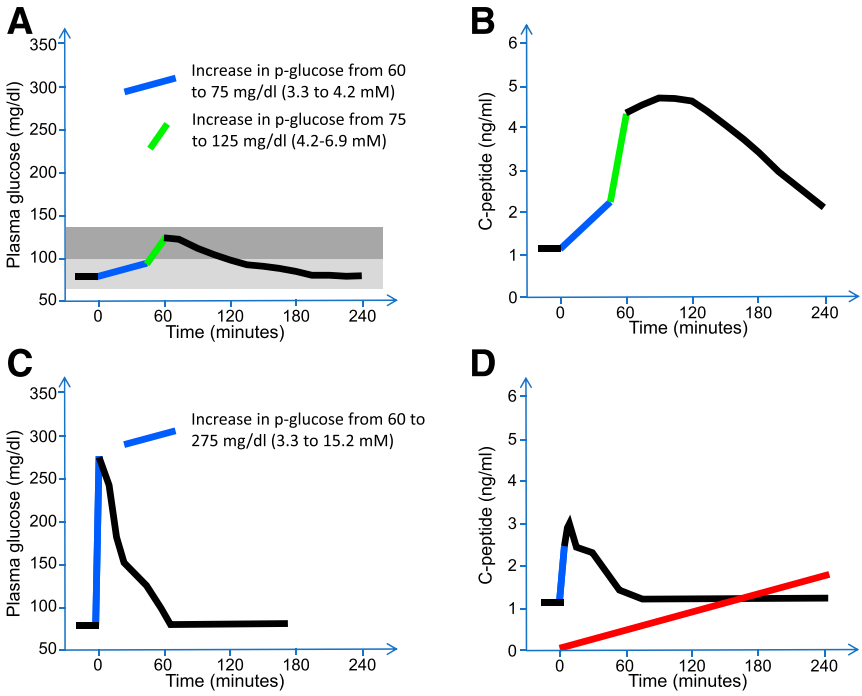

Figure 1—Plasma glucose and corresponding C-peptide levels in response to an oral SM (600 kcal mixed-nutrient meal) (*A* and *B*) or during an IVGTT (0.3 g/kg body weight) (*C* and *D*). Figures are made with values obtained from refs. 10,13,31. CGMS in subjects without diabetes shows that 80% of the glucose values are within the light gray area, and almost all remaining readings are within the dark gray area. During an SM, incretin potentiation allows only a marginal increase in plasma glucose levels (*A*) to induce a massive release of insulin, as measured by plasma C-peptide (*B*). During an IVGTT, plasma glucose is increased to supraphysiological levels within minutes (*C*). Even so, the induced release of insulin, as measured by plasma C-peptide (*D*), is markedly lower than during an SM (*B*). The total stimulated insulin secretion (area under the curve) in response to an SM is about eight times larger than that observed during an IVGTT. The kinetics of insulin release, as measured by plasma C-peptide, from macroencapsulated human islets within the βAir device are shown by the red line in panel *D*. It should be noted that no information on plasma glucose levels after the IVGTT was provided in the study by Ludwig et al. (31), and therefore this information is not indicated in panel *C*.

diabetes. Continuous subcutaneous glucose monitoring (CGMS) on healthy volunteers showed that 80% of all glucose values are 3.3–5.5 mmol/L (light gray area in Fig. 1*A*), and almost all other readings are below 7.8 mmol/L (dark gray area in Fig. 1*A*) (13). Notably, the rise in glucose after breakfast, lunch, or dinner is less than 2 mmol/L (13). In successful cases, clinical islet and pancreas transplantation can achieve a similar level of blood glucose control, demonstrating that normalized glucose metabolism can be achieved with cellular replacement therapies (14,15).

GLUCOSE METABOLISM IN T1D SUBJECTS ON EXOGENOUS INSULIN THERAPY

Despite the availability of novel insulin analogs, glucose sensors, and means of insulin administration, glucose metabolism in subjects with T1D is markedly impaired to that of subjects without diabetes, especially in T1D subjects below the age of 25 (16). Development of late complications and premature death are correlated with the level of blood glucose control (17), and efforts are being directed toward developing novel technologies, e.g., closed-loop systems, as well as toward enabling more patients with T1D to benefit from the technical solutions already available.

A hallmark of all therapies is that the patient "knows" when a meal is to be digested and that exogenous insulin is given in a dose calculated to reflect the amount and composition of the food to be eaten. Although often not perfectly calculated, this dose of insulin is injected at the beginning of the meal, or preferably even 10–15 min before starting the meal, ensuring increased circulating levels of insulin when the digested food is processed in the GI tract and nutrients are taken up in blood, i.e., at a time before the blood glucose levels rise. Given the inherent assumptions in insulin dose decision making, the consequence of avoidance of postprandial hyperglycemia is a later risk for hypoglycemia.

GLUCOSE METABOLISM IN T1D SUBJECTS WITH ENCAPSULATED INSULIN-PRODUCING CELLS

The fact that the only driving force for glucose to enter, and for insulin to leave, an encapsulation device is diffusion has important implications for any encapsulation strategy. Diffusion is critically dependent on the concentration gradient, e.g., the difference in glucose and insulin concentrations in the surrounding intercellular space and those inside the encapsulation device. There is no active transport over the immune barrier. In vitro evaluation of different encapsulation strategies is usually made by changing the glucose concentration in the surrounding medium from 2–3 mmol/L to 16.7 or often 20 mmol/L (18,19). Transplantation of encapsulated insulin-producing cells has been shown to cure diabetes, as evidenced by a rapid return to normoglycemia and normalization of glucose disposal during an IVGTT that, within minutes, increases blood glucose from baseline to about 15–20 mmol/L or higher (blue line in Fig. 1*C*), i.e., levels that are never found in subjects without diabetes (Fig. 1*A*).

Notably, dynamic insulin, or C-peptide release, during the IVGTT has not been determined in most studies on encapsulation (20–28). Even so, normalization of glucose dynamics during an IVGTT has been interpreted as evidence of active insulin secretion, even though it has been known since 1934 (29) that glucose influences its own disposal, independent of changes in the plasma insulin level. In fact, glucose disposal during an IVGTT is normalized in streptozotocin-induced diabetic rats implanted with slow-releasing insulin pellets, in the absence of any capacity to increase plasma insulin in response to high glucose (30). Hence, functional evaluation of encapsulated insulin-producing cells most often occurs in animal models in which glucose effectiveness has the absolutely dominant role in glucose disposal (20–28).

In humans and large animals, evaluation also mainly occurs by creating huge, nonphysiological concentration gradients, forcing glucose into the encapsulation device (31). The blue and black lines in Fig. 1*D* show C-peptide secretion in a subject without diabetes, and the red line shows secretion from high-quality macroencapsulated human islets within the βAir device (31). The kinetics from the βAir device are far from those found in a subject without diabetes, and maximal insulin secretion (measured as plasma C-peptide) is obtained as long as 4 h after the intravenous injection of glucose (31). In contrast, maximal insulin secretion from native islets occurs within only a few minutes after glucose injection (blue and black lines in Fig. 1*D*) (10). This impairment in glucose and insulin kinetics is relevant to all currently available macroencapsulation devices. Furthermore, no data are available demonstrating that insulin and glucagon are released from the device containing encapsulated islets in a pulsatile manner similar to that of native islets (11,12), and future studies should be conducted to address the minute-to-minute dynamics of insulin and glucagon secretion from encapsulated islets to clarify the importance of pulsatile hormone release to allow optimal metabolic control.

The risk for hypoglycemia using an encapsulation strategy has elegantly been shown in experimental studies (32), but is almost negligible when an approach similar to the βAir device is used, mainly because of the low functional capacity of the encapsulated islets. The maximal insulin release obtained with nonphysiological high glucose for an extended period (red line in Fig. 1*D*) is actually lower or comparable with that under baseline conditions in subjects without diabetes (black lines before stimulation in Fig. 1*B* and *D*). It should be noted that βAir device contains only ~150,000 islet equivalents, in part explaining the low, or even absent, absolute values recorded at baseline (31). However, these data also demonstrate the functional capacity of the encapsulated islets during conditions of physiological blood glucose values before the intravenous injection of glucose. The reason for the lack of baseline secretion from the encapsulated islets is at present unknown. It may be due to either the need for diffusion or the low functional islet β-cell mass, or both. However, insulin secretion is

markedly impaired in islets exposed to even a minor reduction in pO_2 (33). Even if the βAir device is constructed to deliver oxygen to the islets via diffusion from a centrally located refillable gas tank (31), the encapsulated islets may be exposed to relative hypoxia because of excessively large diffusion distances (33).

In contrast to the situation in which a subject without diabetes injects insulin before a meal, insulin secretion from encapsulated islets is only triggered by the induced increase in blood glucose. This situation is most likely not compatible with the aim of controlling blood glucose in the normal range without any CGMS readings above 8 mmol/L (13). Thus, as diffusion is the only driving force, the laws of physics prevent insulin release with normal kinetics from encapsulated cells in response to a meal, i.e., high blood glucose levels must first occur to push glucose into the device containing the insulin-producing cells. Subsequently, insulin must accumulate within the device in high concentrations before passive diffusion into surrounding tissues can occur. These limitations on the kinetics of glucose-induced insulin secretion should be considered in relation to the definition of diabetes according to the American Diabetes Association and World Health Organization (34): a subject has diabetes if the plasma glucose is ≥200 mg/dL (11.1 mmol/L) at 2 h during an oral glucose tolerance test. The importance of postprandial hyperglycemia was further emphasized by the American Diabetes Association in their definition of increased risk for diabetes, i.e., subjects with plasma glucose between 140 and 199 mg/dL (7.8 and 11.0 mmol/L) at 2 h during an oral glucose tolerance test (34). The issues of delayed glucose and insulin kinetics with encapsulation devices may be similar, or even worse, when compared with current limitations with closed-loop insulin delivery that does not adequately control postprandial hyperglycemia.

Notably, most materials used for encapsulation today readily allow glucose and insulin to pass immune barriers. Novel materials are therefore expected to only marginally improve the kinetics of glucose-stimulated insulin release as long as diffusion remains the driving force for passage across the immune barrier. Instead, efforts should be directed to further benefit from *1*) minimizing the dead space of the device (the volume not occupied by cells), *2*) enabling the incretin potentiation of insulin secretion, and *3*) ensuring the release of insulin into the portal vein, thereby facilitating the central role of the liver in maintaining near-normal glucose metabolism.

INFLUENCE OF THE DEAD SPACE WITHIN THE ENCAPSULATION DEVICE

Given the risk of a few undifferentiated stem cells remaining within the insulin-producing cells to be transplanted, research is currently focusing on macroencapsulation. These large devices containing several hundreds or thousands of islets are designed to promote surface revascularization in vivo (35,36). Containing the entire cell population transplanted within 1–2 defined containers would allow their removal if teratoma formation occurred. However, the dead space has a huge impact on cell survival and the kinetics of glucose and insulin diffusion. Elegant studies by Colton and colleagues (33,37) showed the importance of the diffusion distance and the dead space in limiting oxygen delivery to encapsulated cells; preferably, the maximal distance from the encapsulated cell to the outer surface of the device should be less than 100 μm, compared with the size of an islet equivalent of 150 μm. Most likely, the maximal diffusion distance for glucose and insulin should be substantially less than 100 μm in order to achieve near-normal kinetics, if only because of the larger size of these molecules in relation to the oxygen molecule. Moreover, in most strategies for encapsulation, the encapsulated cells are immobilized in alginate, further reducing the transport of glucose and insulin within the device (38).

To enhance glucose and insulin kinetics by reducing the dead space, various strategies for microencapsulation have been developed in which each islet is enclosed within a separate container. Thorough studies by Chicheportiche and Reach (38) investigated the influence of the size of microcapsules on glucose-induced insulin secretion. Even islets in capsules with a diameter of 350 μm or smaller showed markedly impaired kinetics for glucose-induced insulin secretion when compared with free islets. Notably, almost no release of insulin was detected in response to 16.7 mmol/L glucose and 5.5 mmol/L theophylline over a period of 30 min from islets contained in capsules with a diameter of ~650 μm (38). These in vitro experiments highlight the importance of the dead space of the capsules and call for approaches involving conformal islet encapsulation, in which the immunoprotective barrier is created directly on the surface of the islets to be transplanted. In theory, this approach would allow glucose-induced insulin secretion almost comparable to that from nonencapsulated islets.

Several novel encapsulation strategies aiming for this level of blood glucose control are now being evaluated in vitro and in vivo (26,39,40). Thus far, this strategy has mainly been implemented by applying a layer-by-layer approach, in which a series of positively and negatively charged macromolecules are added directly to the islet surface. A limitation of this approach is the lack of long-term stability in vivo, with eventual dissolution of the immunoprotective barrier. Scientists using modern "click-chemistry technology" (41) are currently investigating the possibility of creating covalent binding in physiological solutions between the different layers creating the encapsulation barrier. An attractive approach is to take advantage of natural molecules in the human body, e.g., elastin (42,43). The elastin-like recombinamers can be decorated with growth factors or other molecules to promote islet cell survival and revascularization of the surface of the encapsulated islet after implantation (44,45). The covalent binding induced between the various layers would provide stability and longevity. These nm-thick layers of islet-adherent encapsulation, decorated to exert crucial biological functions, hold great promise for future replacement therapy.

Moreover, islet-adherent encapsulation allows transplantation approaches similar to those already developed for clinical islet transplantation, such as intraportal infusion.

INCRETIN EFFECT AND INFLUENCE OF THE SITE OF IMPLANTATION

Incretins like GLP-1 exert their potent biological effect at picomolar concentrations, i.e., at ~1/100,000,000 of the blood glucose concentration, and with a half-life of only a few minutes (46). These basic physiological considerations make it highly unlikely that a concentration gradient large enough to drive GLP-1 into an encapsulation device will be created. Tentatively, systemic delivery of GLP-1 analogs or DPP-4 inhibitors circulating in blood with half-lives of hours to weeks (47) would allow saturation of the incretin receptors on the encapsulated insulin-producing cells.

Formation of blood vessels and a capillary network close to the encapsulation device is mainly driven by the local inflammation induced at the time of implantation and, to some extent, also by factors released from the hypoxic cells within the device. Ambitious screening studies have been conducted in order to identify materials that allow the formation of capillaries close to the immunoprotective barrier of the device (36). However, almost no studies have described the level of blood flow through these capillaries. The small amount of information available shows only a limited microcirculation around the device at the time of implantation, with no further increase over a period of 12 months (48). Regulation of blood flow is highly complex and, to a large extent, dependent on the site of implantation. Blood flow to an encapsulation device will most likely not be associated with an intrinsic blood flow regulation but instead be regulated in a manner similar to that of the implantation site. In contrast, free cellular islet transplants, without encapsulation, develop an inherent site-independent blood flow regulation; however, this occurs only as much as 12 months or more after implantation (49). Also, reinnervation that may contribute to turning off insulin secretion in response to declining blood glucose is prevented by the encapsulation barrier.

For logistic reasons, the most attractive site for large-scale application of an encapsulation strategy as a future cure for T1D is the subcutaneous space. However, the subcutaneous tissue has low blood perfusion, and, most importantly, this perfusion is kept at an absolute minimum whenever the ambient temperature is low or if there is a cold wind blowing in the face of the subject studied (50). These ordinary physiological considerations have immense implications for the choice of the site at which encapsulated islets should be implanted to allow a constant high blood flow in the surrounding tissues, which is of utmost importance for the kinetics of glucose-stimulated insulin secretion.

Encapsulation of insulin-producing cells generated from stem cells has inherent safety concerns, and the cells should therefore be implanted at a site from which the encapsulation device easily can be retrieved if problems occur. However, these safety concerns should be carefully balanced by the physiological considerations discussed above that must be considered in order to allow survival and optimal function of the implanted cells.

DEFINING WHAT IS "GOOD ENOUGH"

Preventing rejection and demonstration of morphologically intact allogeneic insulin-producing cells in humans after encapsulation constitute major scientific achievements (31). Even so, these achievements are not predictive of our future capacity to create near-normal glucose metabolism in a patient with T1D, as glucose and insulin passage across the immune barrier depend on passive diffusion (31).

Considering the basic physiological and physical laws discussed above, we must define what is "good enough" for a cellular replacement therapy aiming to cure T1D. Continuous release of insulin from the encapsulated cells is obviously not good enough. An encapsulation strategy must provide benefits superior to the regulation achievable from closed-loop insulin, or insulin and glucagon, delivery systems. Preferably, insulin and glucagon release should be pulsatile in order to allow close-to-normal physiological glucose metabolism. As long as the encapsulation strategy depends on diffusion, high blood glucose levels must occur before insulin is released from the encapsulated cells: inherent in even the theoretically best systems is a considerable delay in insulin secretion that will cause postprandial hyperglycemia potentially followed by later reactive hypoglycemia.

In order to prevent late complications and premature death, the encapsulation approaches discussed above may not, however, be good enough. The risk of myocardial infarction in subjects with type 2 diabetes with postprandial hyperglycemia $\geq$10 mmol/L is increased by 40% when compared with subjects with postprandial glycemia $\leq$8 mmol/L (51). This increased risk of myocardial infarction in subjects with diabetes can be reduced to the levels of the general population if postprandial hyperglycemia is reversed. Thus, it is likely that an encapsulation strategy in addition to producing near-normal glucose metabolism with normalization of HbA_{1c} also should prevent postprandial hyperglycemia in order to provide clinically relevant benefits when compared to what can be achieved with modern insulin therapy. The choice of any replacement for T1D should follow a careful risk and benefit assessment, especially considering the preferential target of subjects undergoing such treatment, i.e., patients with brittle diabetes and severe risk of fatal hypoglycemia. The answer to the question "what is good enough" cannot be simply defined based on glycemic control but should be patient oriented, depending on the indication and the defined goal for the treatment.

CONCLUDING REMARKS

The insulin-producing cells within the islets are among the human cells with the highest cellular metabolism. The islets of Langerhans are among the tissues in the human body that are most highly perfused with blood, and each insulin-producing cell is in direct contact with a blood

capillary. This arrangement has most likely evolved in order to ascertain metabolic support and control of blood glucose within strict physiological limits.

Free cellular transplants, in contrast to encapsulated islets, become revascularized and integrated with the surrounding tissues (49). Experience from clinical islet transplantation shows proof of principle that cellular replacement therapies in subjects with T1D can control blood glucose within the normal range (14,15).

The immune response to an allogeneic cell is well characterized and mainly depends on differences in HLA between the donor and the recipient. The huge variation in human HLA is illustrated by the 25–50% failure rate in identifying an HLA-identical unrelated donor for allogeneic hematopoietic stem cell transplantation among the 25 million HLA-typed healthy volunteers listed in databases worldwide (52). Modern genetic techniques provide an elegant, and thus far almost unexplored, way of circumventing the HLA barrier and allowing transplantation without the need for systemic immunosuppression (53,54). A stem cell line, human embryonic stem cells or induced pluripotent stem cells, could be genetically modified by removal of all HLA alleles except one, e.g., HLA A2. Such a stem cell line will likely escape natural killer cell–mediated killing, as it will be recognized as "self" because of the HLA allele expressed. If so, transplantation of this particular cell line (HLA A2) can be performed in about 30% of the Caucasian population without considering mismatches with regard to additional HLA alleles. Creation of ~10–20 such cell lines would allow cellular replacement therapies in almost all subjects with T1D without the need for systemic immunosuppression, as HLA matching would be performed for only one HLA allele instead of the multiple HLA alleles that are routinely necessary for finding a HLA-identical donor for hematopoietic stem cell transplantation.

This future scenario holds promise for all subjects already affected by T1D; however, this approach also carries several inherent risks that must be addressed, such as recurrence of autoimmunity and inadequate immune control of virally infected or transformed cells in the product. However, a macroencapsulation strategy could make possible the initial clinical trials aiming to obtain safety results on stem cell lines in humans, the advantage being that the device containing the cells can readily be explanted if problems occur. This approach could also be used to allow in vitro analysis for safety and functional characterization after defined periods in the human body. Assuming that safety can be demonstrated, subsequent clinical trials aiming for efficacy and cure of T1D would likely be performed with conformal microencapsulation or using free cellular grafts genetically modified to escape rejection.

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