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# Reversing Type 1 Diabetes: The Magnifying Potential of Stem Cell Encapsulation

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## Abstract

Type 1 diabetes (T1D) is one of the most common chronic autoimmune diseases characterized by islet autoimmunity. This is followed by immune destruction of the  $\beta$  cells as T cells attack and destroy insulin-secreting pancreatic  $\beta$  cells, leading to insulin deficiency. Currently, life-long insulin therapy is the primary treatment option for the condition with research being centered around islet transplantation to restore glycemic stability. However, this procedure is limited by risks and supply shortages, highlighting the need for a safer, more effective therapy for the approximately 9 million people across the world with type 1 diabetes. This literature review assesses stem cells and their potential as a vast  $\beta$  cell supply towards the treatment of type 1 diabetes. Based on current findings, stem cells may differentiate to become a self-renewing  $\beta$  cell line that may reverse type 1 diabetes; however, further studies expanding on ncapsulation techniques, methods whereby living cells are entrapped in semi-permeable membranes for the purpose of disease treatment, are required. With this new horizon of possibilities, targeted efforts towards stem cell manipulation in expressing  $\beta$  cell phenotype can pave the way for a high efficiency treatment for type 1 diabetes.

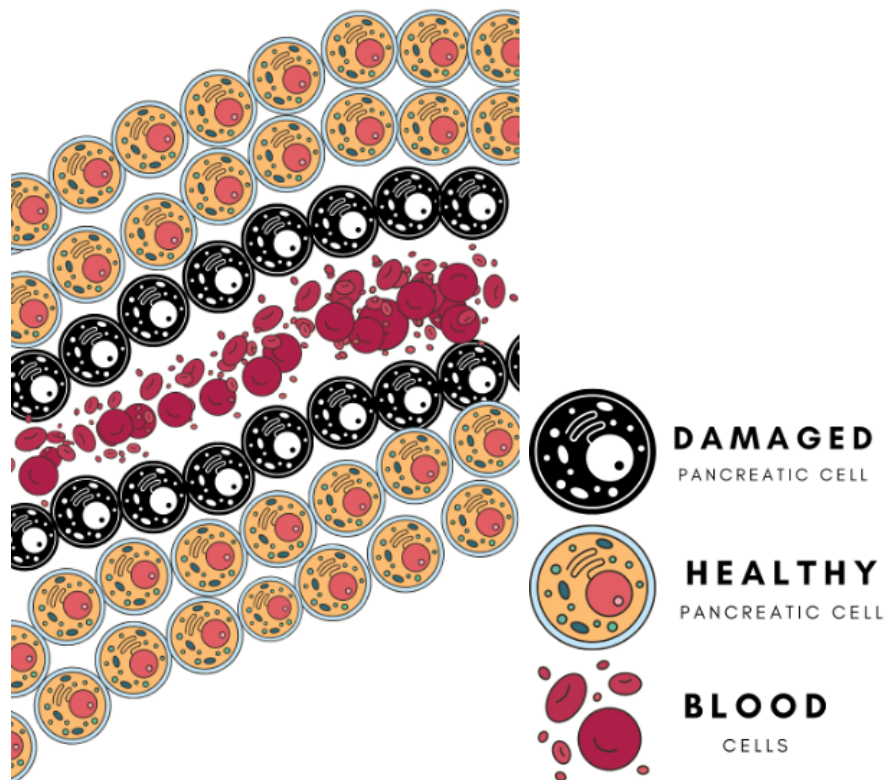
## Introduction

Type 1 diabetes typically has a sudden onset during childhood or early adolescence, often diagnosed when the child visits the hospital for one of the condition's symptoms. These symptoms include polyuria, increased thirst, blurred vision, and weight loss<sup>1</sup>. If not diagnosed within the first few weeks, diabetic ketoacidosis (DKA), a condition in which excess blood acids are produced, may develop<sup>2</sup>. This condition is often fatal if left untreated and is marked by abdominal pain, confusion, and nausea. Alternatively, hypoglycemia, a condition in which the patient's blood glucose levels are low, is caused by excess insulin, low levels of eating, or excessive gaps of time between meals<sup>3</sup>. Hypoglycemia and diabetic ketoacidosis are often caused by missing insulin shots, especially if T1D is still undiagnosed. T1D, diabetic ketoacidosis, and hypoglycemia may all be diagnosed through a urinalysis or blood test that reveals abnormally high levels of glucose and blood acids.

The onset of T1D is typically sudden, making early diagnosis and treatment integral. Stage 1 displays no symptoms, even when a patient's hemoglobin A1C, a blood test for prediabetes and type 2 diabetes, is tested<sup>4</sup>. However, antibodies have already begun to destroy insulin-producing cells. Left unnoticed, stage 2 characterizes itself with increased  $\beta$  cell loss and hence abnormal blood glucose levels as a result. Though symptoms may still not appear, the antibodies' attacks have, at this stage, led to pancreatic damage<sup>4</sup>. It is at this point, stage 3, where symptoms begin due to the great loss of  $\beta$  cells. Though the direct causes of these stages are still unknown, certain patients appear pre-disposed to the later stages.<sup>4</sup>

While direct causes of T1D are still unknown, some risks have been identified. Environmental risks, such as cesarean section births or the time in life when cows' milk is introduced into a child's diet, have been associated with the development of type 1 diabetes<sup>5</sup>. A popular theory linking the environment to T1D links early-life viral infections to the condition's development<sup>5</sup>. Genetics have also been proven to partially cause type 1 diabetes, as the risk of developing the condition ranges between 1%-70% depending on one's genetic proximity to a family member with type 1 diabetes<sup>5</sup>. Additionally, certain drugs and medications can damage  $\beta$  cells, reducing the production of insulin and causing a condition very similar to

type 1 diabetes<sup>5</sup>. In some particular cases, such as a rodenticide introduced in America in 1976 by the name of Prynuron, the medication can induce the destruction of pancreatic  $\beta$  cells, hence leading to type 1 diabetes<sup>6</sup>. Prynuron was withdrawn from the American markets after just 3 years; however, other drugs may still lead to pancreatic inflammation. These risks for and causes of T1D are in need of further research because, as of now, there is no cure for type 1 diabetes.



*Figure 1: Damaged cells are unable to create insulin.*

Previous studies have shown that stem-cell therapy may be developed into an efficient form of cell therapy for Type 1 Diabetes<sup>7</sup>. The goal of this article is to comprehensively review and outline the most recent data surrounding the benefits, risks, and details of stem-cell therapy as it relates to Type 1 Diabetes treatments. Even though treatments have advanced and the quality of life of T1D patients has improved, T1D prevails as a widespread issue.

Currently, the most prevalent treatments include insulin injections, continuous glucose monitoring devices, utilization of glucose tablets as needed, and a general healthy lifestyle<sup>1</sup>. As widespread as these options are,

they hold great drawbacks. Insulin injections are a daily maintenance where patients inject insulin subcutaneously in the area between the skin and muscle, typically a location with enough adipose. If injected deeper by mistake, low blood glucose levels combined with increased pain typically occurs. Furthermore, injection sites must be rotated to avoid lipodystrophy, a condition where adipose causes indentations that interfere with insulin absorption<sup>8</sup>. Continuous glucose monitors, in contrast, involve inserting a minuscule sensor under the skin that measures interstitial glucose levels every few minutes<sup>9</sup>. The data is sent to a monitor, which is sometimes a part of the insulin pump, for the patient to examine when needed. While this option minimizes technically difficult self-administered shots, it is limited by a high cost that makes the device inaccessible to a large portion of the Type 1 population. Healthy lifestyles and glucose tablets may be used in combination with traditional blood pricks and glucose monitoring. However, these are not cures, nor do they reverse the effects of T1D. Due to the prevalence of this disease, more efficient treatments and therapies are demanded.



*Figure 2: Current popular type 1 diabetes treatments.*

Stem-cell transplantation is an encouraging procedure in both cost and safety. Stem cells are marked for their capacity to differentiate into different

types of cells, creating a large supply of any of the 200+ types of human cells<sup>10</sup>, including bone marrow cells, red blood cells, nerve cells, and most importantly for our purposes, islet cells. Hematopoietic stem cells, the primary adult stem cells used in medical settings, are found in bone marrow and are utilized to form blood cells<sup>11</sup>. This allows for stem cells to be used in integral procedures like bone marrow transplants and holds promise for stem cells to be developed into other cells for other procedures. These unspecialized adult stem cells (AsCs) can develop into islet cells for islet cell procedures<sup>12</sup>. Our goal has been to examine this potential procedure that may be the key to a cure of type 1 diabetes.

Pancreatic cell transplantation is able to stabilize blood glucose levels on its own, however, there is a chronic, nationwide shortage of donors and immunosuppression therapy<sup>13</sup>. Islet cell transplantation is an alternative that is less invasive than pancreas transplantation and also proves to be effective in reversing complications from T1D but has similar limitations to pancreas transplantation, still suffering a lack of donors and hindering immunosuppression therapy. AsCs and embryonic stem cells (ESCs) can be differentiated into pancreatic islet-like cells to produce insulin in response to change in blood glucose levels<sup>14</sup>. This alternative is cost effective and involves no donor shortages due to the self-producing cell line. However, transplantation is a potential issue. Sourcing of human stem cells requires exploring as new research heavily focuses on umbilical cord stem cells rather than AsCs. Additionally, immunosuppression when stem cells are implanted is still unclear, leading to the potential benefits of encapsulation devices. Current strategies for this involve implanted cells shielded from the immune system by a physical barrier. Encapsulated stem cell-derived islets may shield  $\beta$  cells from the immune system, ensuring an almost limited supply for islet cells in procedures to cure type 1 diabetes<sup>15</sup>. However, encapsulation device strategies need to be improved in order to minimize foreign body response, possibly by targeting different sites. Moreover, transplantation of stem cells through encapsulation in minimally invasive areas is still being investigated. Nevertheless, this research emphasizes the possible benefits within stem-cell therapy based T1D treatment and highlights its need for future research.

## Current Cell Therapies

Current cell therapies in use hold significant value as they can guide scientists to potentially more efficient and cost-effective alternatives. Stem cell therapies today include regenerative medicine that causes repair response. A major difficulty within these is that organ donors have to fit restrictive criteria. The different cell types, such as totipotent, pluripotent, and embryonic stem cells, allow for a variety of treatments suited for appropriate diseases<sup>16</sup>. It is of utmost importance to carefully select the type of stem cells that are suitable for clinical application.

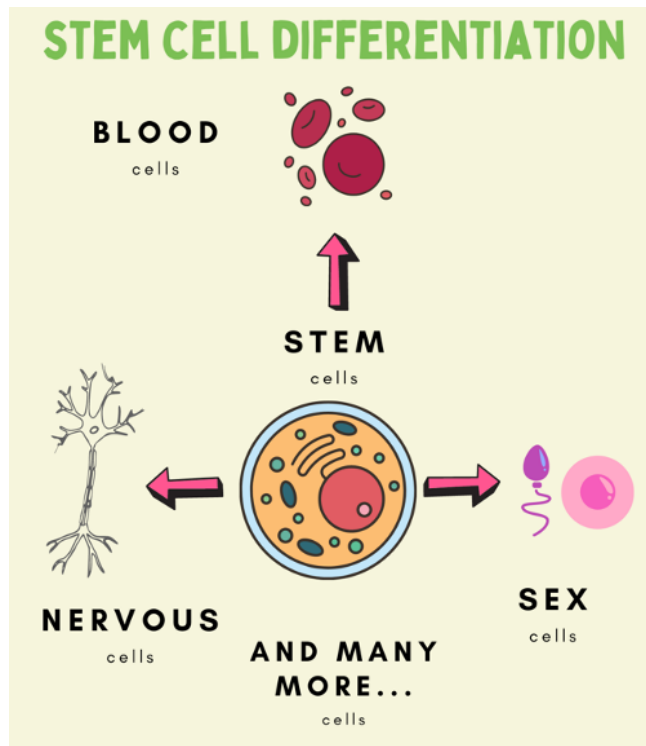
Specifically pertaining to type 1 diabetes, islet cell transplantations, where islets are taken from the pancreas of an organ donor, contain beta cells that produce insulin and have been used as a treatment for years now. Naturally, with this treatment, difficulties such as limited supply of human islets and poor immunosuppression arise<sup>17</sup>. Other potential treatments to reverse T1D involve Mesenchymal stem cells (MSC), whose self-renewal potential and ability to differentiation into functional cell types can cure diabetes<sup>18</sup>. Potent strategies combatting these transplantation difficulties have been explored: alternative transplantation sites, novel immune protective agents, and encapsulation techniques. A clinical trial currently run by the University of Alberta beginning 2021 evaluates pitfalls of islets but also solutions to islet cell transplantation<sup>19</sup>. This highly beneficial search for higher efficiency and safety as it pertains to current cell therapies is a major factor as to why islet cells are a great therapeutic treatment method but also why stem cells may be a better option.

Furthering the MSC treatment plan, one stem cell-based clinical trial for Diabetes Mellitus is the intraportal allogeneic cadaveric islet transplantation. Due to the lack of coverage for transplant costs along with the limitations of cadaveric islets, alternatives have been sought, specifically regarding Mesenchymal stem cells. As a source for newly generated beta cells, such cells have been proven to be effective on type 2 diabetes. However, this is not the case for type 1 diabetic patients, as Mesenchymal stem cells cannot differentiate into beta cells as effectively in vitro. In vivo, this differentiation does not occur at all. Instead, human embryonic stem cells are a plausible treatment method that are being studied as surrogates in replace of cadaveric

islets. Immune rejection however is a specific issue to this current cell treatment that can be addressed. One potential research route, which will be explained in more depth later on in this article, is the ability to couple hESC derived organoids that produce insulin with microencapsulation technologies. This optimizes the need for vascularization and can create a more beneficial route in reversing type 1 diabetes<sup>20</sup>.

This research continues to advance in order to ensure safe therapy, as well as develop efficiency in utilizing adult stem cells, including bone marrow transplants of hematopoietic stem cells. Specific future research targets involve in vitro studies on the production of functional stem cell-derived  $\beta$ -cells and how they respond to glucose. This will demonstrate how cells respond to different forms of beta cell stress. The protective mechanism exhibited by suppressive immune cells in the pancreas shows promise for future stem cell techniques and potential target sites. Similarly, human pluripotent stem cells (induced pluripotent stem cells) serve as alternative beta cell sources for transplantation when there are donor shortages for other treatments. Another example of current/pursued research: beta cell replacement through the transplantation of islets of Langerhan.

The figure below details stem cell differentiation, the process by which stem cells form more specialized functions through signaling mechanisms like DNA methylation. The signaling mechanisms are transmitted through nerve cells which generate electrical and chemical signals of action potentials and neurotransmitters to send information. Blood cells are one type of specialized cells that can be derived from stem cells, whose specialized function include its self-renewal potential. In a study published in 2015, the generation of sex cells through stem cell differentiation is explained, providing new procedures for the efficient generation of such cells from embryonic stem cells, a specific stem cell discussed later. In the study, mouse embryonic stem cells are signaled to differentiate into Epiblast-like cells and finally to PGC like cells, or primordial germ cells, a precursor to all germline cells. The versatility of stem cells in differentiating into many key specialized cells make them optimal for transplantation as it pertains to diabetes, as the self-renewal potential and personalization of the treatment can prove to be more effective and beneficial<sup>21</sup>.



*Figure 3: Stem cell differentiation.*

Limited donors comprise a portion of the difficulties with stem cell treatments, but economic cost also plays a large role. For example, even costs to create beta cells from stem cells are similar to the cadaveric islet method. Both mechanisms, though useful and still developing, require more money, and still immunosuppression issues and autoimmune rejection remain a major factor in their inefficiencies. So far, only a handful of trials have used human embryonic stem cells in order to regenerate beta cells. While current cell therapies include wearable insulin delivery devices made possible by modern therapy's increasing normoglycemic ranges—a significant improvement over regular insulin pumps—these are not stem cell therapies and are no closer to reversing the effects of T1D. Rather, the ability to use stem cells to enhance treatment by coupling it with other alternatives like macro or micro encapsulation can prove to be much more beneficial in the long run.



## Combined Stem Cell Alternatives

Current stem cell therapies have been combined with other studied therapies and/or biomaterials to explore the ability to heal the effects associated with T1D or provide reversal treatment altogether. One combined stem cell therapy that has been explored to heal diabetic wounds is through treatment with human umbilical cord-derived mesenchymal stem cell-derived exosomes (hUCMSC-exos) and Pluronic F-127 (PF-127), which is a medicating hydrogel. PF-127's unique thermal properties and porous structure allow for the release of therapeutic proteins, hypothesizing that PF-127 can continuously release hUCMSC-exos directly onto T1D-affected tissues, thus attracting fibroblasts and endothelial cells to initiate wound repair<sup>22</sup>. This treatment was explored through topical application as this delivery was easy, convenient, and non-invasive, with high-efficiency and low toxicity.

The treatment was tested by applying the exosome-hydrogel combination on diabetic rat models, and the researchers observed angiogenesis, cell proliferation, and granulation tissue formation to understand the capabilities of this wound repair mechanism. The diabetic rats were tested in three different groups; one group was treated with hUCMSC-exos only, one with PF-127 hydrogel only, and the last with the combination treatment of hUCMSC-exos/PF-127. It was found that after 7 days the wound area was significantly smaller in the combination groups versus the others, and by day 14 the wounds were completely healed for this group<sup>22</sup>. Combination treatment with hUCMSC-exos and PF-127 hydrogel allows for the enhanced survival of exosomes and for a controlled release on wound tissue over time which shortens the wound healing time. This study is a notable example of how combined therapies can result in enhanced treatment and accelerate healing time.

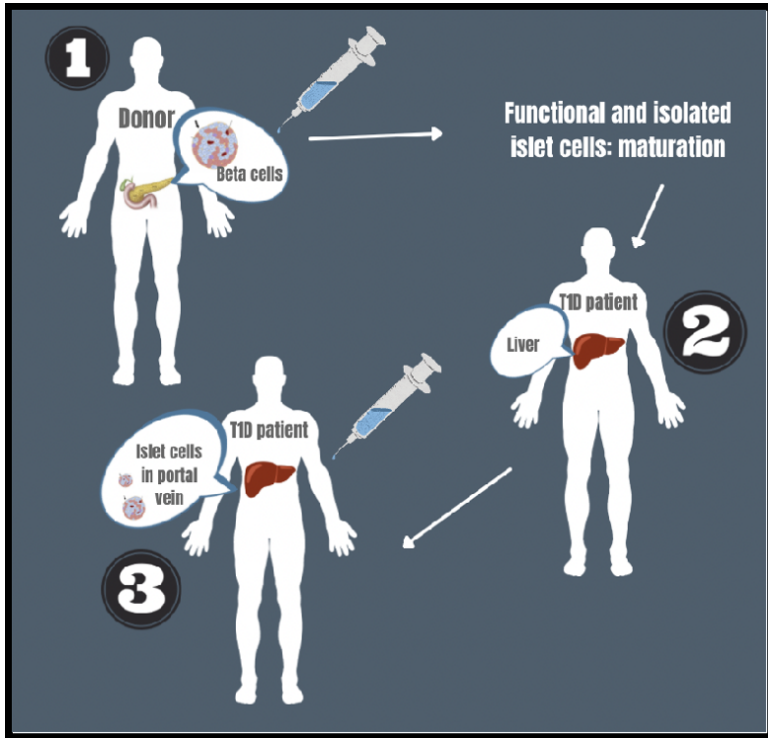
Further alternatives have been explored using insulin producing cell therapies based on stem cells and combined transplantation with Mesenchymal stem cells. Studies have shown that though MSCs prove to be ideal cellular sources due to properties relating to tissue repair and immunomodulatory capacities, further clinical trials have expanded on the idea that its properties are not as effective as expected. Hence, a study

combining MSCs and human Type 2 Diabetes islets ex vivo and in vivo was performed exploring reverse beta cell dedifferentiation<sup>5</sup>. The combination of MSCs and islets presents a tempting alternative treatment, particularly as adult MSCs suffer fewer ethical issues pertaining to self-renewal and differentiation capabilities. Although this alternative has been explored with Type 2 Diabetes islets rather than Type 1, it offers a novel strategy to reverse dysfunctionality of beta cells, which is crucial given that they secrete insulin.

Coupling embryonic stem cell therapy with macro- and micro-encapsulation devices has the potential to balance the necessity of immune protection. This may serve as an innovative strategy to reverse T1D and in the future may be used to overcome the issue of hosts' immune responses, improve immunoengineering strategies and encapsulation technologies. A study involved the transplantation of human embryonic stem cell-derived pancreatic progenitors in macroencapsulation devices into diabetic mice. It aimed to create an improved differentiation protocol to prevent forming excess tissue in places that weren't targeted, mainly the mesoderm. This method explored how variations of environments could influence in vivo pancreatic progenitor development. Such cells differentiated into pancreatic endocrine tissue in macroencapsulation devices, resulting in a reversal of diabetes within 3 months in the mice<sup>23</sup>. This process proved to be successful in generating grafts, necessary to fuel islet cells, in greater than 80% of the endocrine cells. Furthermore, 99% of tested mice did not show signs of formed non-endodermal cell populations, providing evidence that an efficient differentiation of human embryonic stem cell-derived pancreatic endocrine cells has potential to couple with a macroencapsulation device, even without direct contact with host environment, ultimately making it capable of reversing diabetes effectively.

The figure below illustrates the transplantation possibilities of functional and isolated islet cells in maturation. In a study on the recovery of beta cell deficiency in type 1 diabetes, it was found that the beta cells regeneration is due to endogenous regeneration or exogenous supplementation. This means that transplantations of certain islets or grafting new beta cells from in vitro cell engineering is a potential alternative through transplantation of islets. Mice models have been used through transgenic expression to study

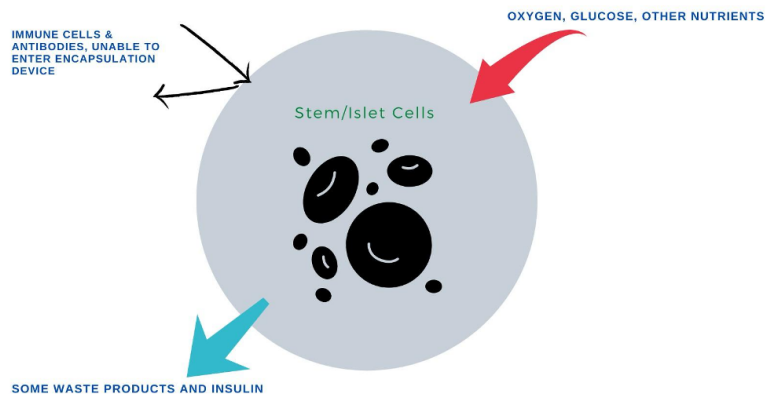
inducible and reversible beta cell destruction. Ultimately, studies on immunological mechanisms related to T1D and novel treatment strategies such as grafting beta cells in vitro can prove significant in finding potential type 1 diabetic drug targets for future clinical trials<sup>24</sup>.



*Figure 4: Islet cell transplantation.*

## Encapsulation Techniques

### CELL ENCAPSULATION-PANCREATIC-TYPE CELLS



*Figure 5: Cell encapsulation.*

## I. Macroencapsulation

Macroencapsulation devices can assimilate islets into semi-permeable membranes that elude typical immune responses while simultaneously allowing for transplanted cells to transport insulin. Type 1 diabetes occurs due to an autoimmune response which attacks insulin producing beta cells, yet the complications of immune rejection of islet-like stem cells can be prevented with macroencapsulation devices. Immunoprotection is achieved by the selectively permeable membrane that impedes the movement of immune cells and immunoglobulins into the device, while allowing the free diffusion of oxygen, nutrients, insulin, and glucose to and from the encapsulated cells. Stem cells are contained within a compartment of the device which allows for selective exchange of nutrients and obstructs antibodies from entering<sup>25</sup>.

When considering macroencapsulation as a potential therapeutic for reversing T1D, it is also crucial to consider implantation sites and shape optimization to maximize the volume of the device within a space. A recent study tested the posterior rectus sheath plane (PRSP) as a potential implant site to host the macroencapsulation device. This plane is in between the muscle belly and the fascia of the rectus abdominis muscle and this site is being explored as implantation and retrieval can be performed without invading the peritoneal space. PRSP has a large blood supply, allowing for greater diffusion of nutrients, and the encapsulated cells therefore receive adequate amounts of oxygen. In order to maximize the space in the PRSP, the best shape for a macroencapsulation device here is presented to be a polygonal-shaped device. These polygonal shapes are ideal as they deliver significantly more cells as compared to device shapes such as circles or rectangles. Although a polygon-shaped device presents the most ideal solution, the sharp angles pose challenges with manufacturing and patient comfort. The polygonal shaped devices have favorable interactions with the surrounding environment, and the implantation within diabetic pigs has shown to be a minimally invasive procedure, but the long-term performance of these devices remains untested<sup>26</sup>.

Aside from optimizing the site-specific sites, a prominent problem with macroencapsulation is being able to supply the encapsulated cells with

enough oxygen. In a clinical trial published in 2018 studying the encapsulation sites for optimal delivery of insulin, the  $\beta$ Air device was developed to overcome this obstacle<sup>27</sup>. The device contained allogeneic human pancreatic islets and was implanted into 4 diabetic patients. Two key sites that ensure easy access to minimal surgical intervention are the pre-peritoneal cavity and under the skin. The significance of these sites and the overall use of the  $\beta$ Air device is to ensure retrievability and immunoprotection. The results of the trial provided evidence that such a device that utilized macroencapsulation was indeed safe and capable of preventing rejection of implanted cells. However, metabolic control was impacted with the transplanted cells' limited function. Potential claims for the inefficiency of the transplanted cells include hypoxia and hyperoxia which could contribute to a lesser device volume, which is undesirable when needing to deliver insulin and nutrients at a productive rate.

Macroencapsulation devices must support viability of the transplanted cell at all stages through the maturation process. These devices are beneficial as they allow for immunoprotection of transplantation islet-like stem cells and can also be retrieved with ease in any circumstance. One limitation present with macroencapsulation devices is accessing a space for implantation. The mechanics of the device are acted upon by different external forces depending on where implantation occurs, which can in turn limit the functionality and lifetime of the device. Although recent studies have begun exploring new sites and have been able to successfully implant islet-like stem cells in microencapsulated devices in a minimally invasive fashion- the PRSP is a great example of this type of site. Another major limitation is that islet cell survival heavily depends on the supply of oxygen; this is affected by the devices membrane permeability of oxygen, the rate of oxygen consumption of the encapsulated islet cells, and other factors as well<sup>28</sup>. One strategy that has been considered to overcome any oxygen deficiency that SC-islet cells may encounter is oxygen delivery to encapsulated cells through oxygen generating materials. In situ oxygen supplementation with the use of an oxysite disk being placed within the center of the macroencapsulation device has been shown to provide adequate oxygen supplementation and improve the survival of cells<sup>29</sup>.

Unlike macroencapsulated devices, microencapsulated devices avert vessel ingrowth, limiting the supply of nutrients to solely diffuse through the selectively permeable membrane. This may also result in a hypoxic environment and therefore requires the need for oxygen delivering technology within encapsulation devices.

## **II. Microencapsulation**

Microencapsulation involves small islet cell mass which favors diffusion of humoral factors and molecules from inside of capsules to the outer environment. The devices are made of thin polymer films which tightly adhere to individual islet or cell clusters. They occupy a very limited graft volume and could be eligible for alternative graft sites. Issues may still arise from efficiency of immune barrier competence, the site of implant, and dependence on the final microcapsules size. While conformal microcapsules fit a wide array of potential graft sites due to their small size, the issue of their long-term endurance arises. Furthermore, microencapsulation requires an adequate oxygen and nutrient supply despite the decreased level of invasiveness regarding the site of the microencapsulation transplantation.

In a research paper focused on microencapsulation devices, researchers conducted a clinical trial to understand how an acquired immune tolerance in patients with type 1 diabetes could prevent an autoimmune attack of pancreatic islet beta-cells. Researchers studied G3C hybridoma triggering the glucocorticoid-induced tumor necrosis factor receptor-related costimulatory receptor (Gitr) to determine if this would promote expansion of Tregs<sup>16</sup>. The G3C monoclonal antibodies were enveloped in microcapsules which were engineered to allow selective flow of immunoglobulin M. In the end, researchers observed that long-term Gitr triggering did induce Treg expansion and prevent diabetes from development in NOD mice- this clinical trial shows promise that microencapsulation can be a possible treatment for autoimmune diseases.

A study conducted in 2019 observed co-microencapsulation of human umbilical cord-derived mesenchymal stem cells (hUCMS) and pancreatic islet-derived insulin producing cells (hIDC), very similarly to what this review proposes as a solution to reversing type 1 diabetes. NOD mice were

grafted with microencapsulated hUCMS and hIDC and observed for 180 days. By the end, general health of these mice improved with signs of increase in body weight and the cell co-aggregates were still intact and viable, which proves that there was a sufficient exchange of nutrients<sup>30</sup>. The microcapsules were easily retrieved, which dismisses one of the main limitations of microencapsulation techniques. Once retrieved, there were no signs of pericapsular fibrotic overgrowth, and insulin and glucagon were still expressed. This study has shown that co-microencapsulation of hUCMS/hIDC is successful and can offer a new route of cell therapy of type 1 diabetes. Considering the co-microencapsulation was successful through this study, it is likely that microencapsulation of only human mesenchymal stem cells (hUCMS) would also provide promising results for reversing type 1 diabetes.

Microencapsulation is a viable method of encapsulating cells as it eliminates the need for immunosuppressant medications by shielding cells from an attack through the host's immune system. The three-dimensional microcapsule has a semipermeable membrane to allow for the exchange of nutrients and waste, creating an optimal environment for stem cells to survive. One major limitation of microcapsule transplantations is pericapsular fibrotic overgrowth due to a foreign body response, yet research has shown that the use of a chemically modified alginate derivative can be effective in the prevention of overgrowth as it works to limit movement and clumping. The study conducted with co-microencapsulation mentioned earlier also proved that microencapsulation techniques are viable and can be retrieved without overgrowth occurring. Although pericapsular fibrotic overgrowth is a major limitation for the long-term effectiveness of microencapsulation, few solutions have been found to overcome this obstacle.

### **III. Encapsulation Sites**

Diabetic treatment using encapsulated islet cells has continued to grow with new devices such as the aforementioned macroencapsulation and microencapsulation. The importance of finding a clear and effective site is to allow for the efficient glucose level regulations in an automated, continuous manner. Several studies have sought an appropriate site for transplantation

that limits the foreign body immune system response transplantation. For example, the intravascular macroencapsules are particularly integral in achieving a connection between solute transport and diffusion of nutrients<sup>31</sup>. Some appropriate sites may include those in the vascular system and may include shunts as well as diffusion chambers.

Finding an efficient site limits any detrimental impact of foreign body immune response. Such responses are a product of microencapsulation and macroencapsulation sites, and their severity can include inflammatory responses and hypoxia. Hence, many factors play a role in determining adequate sites such as material aspects on porosity, roughness, size of implant, and surface charge. Current sites that are explored, however, are generally limited in their space in renal capsules conventional sites, and the respective volume of such sites is necessary in order to create a long-term treatment of such encapsulation devices rather than a temporary solution.

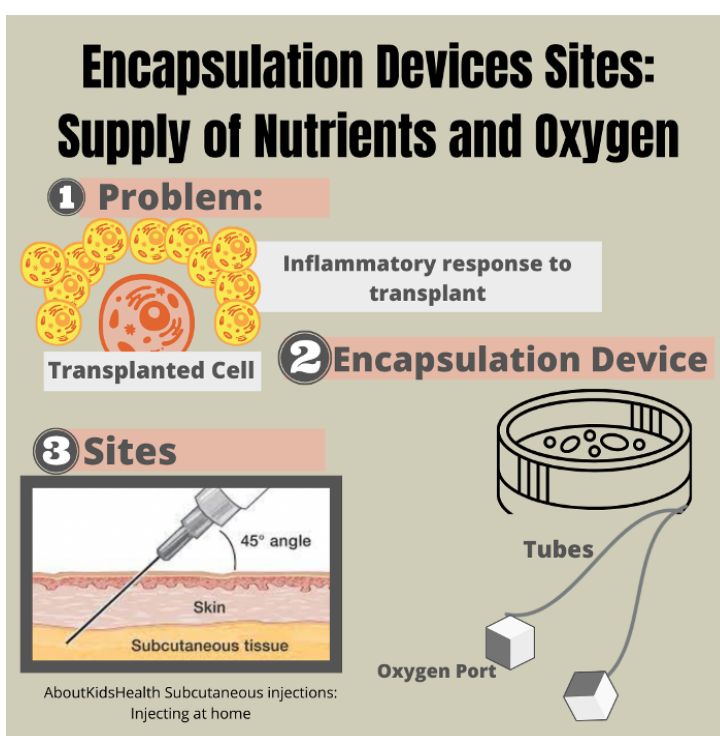
Microencapsulation sites are ideal as they have increased ease of access for implantation, retrieval, and imaging. Furthermore, there is a sufficient blood and oxygen supply. The encapsulation site is still limited as the only site that has been explored so far is the peritoneal cavity for implantation. Other implantation sites that are being explored include epididymal fat pad, skeletal muscle, and subcutaneous tissue. However, transplantation in these alternate sites remains an obstacle.

Macroencapsulation sites are large transplant masses within a single, well-defined 3D device, allowing for device retrieval in case of adverse reaction or failure. Most are implanted within the peritoneal cavity and subcutaneous space. While the peritoneal cavity site of microencapsulation allows for intrinsic high vascularity and oxygen tension, graft implantation and monitoring generally requires invasive procedures. Subcutaneous space transplantation is less invasive and also provides adequate oxygen which facilitates cell survival.

A site for both macroencapsulated and microencapsulated islet-like stem cells must be in close contact with the bloodstream for a sufficient supply for nutrients, yet the liver and spleen (which typically host nonencapsulated



islets successfully) are unable to tolerate the large volumes that are associated with encapsulation techniques<sup>32</sup>. Due to less invasive procedures, most transplantations of encapsulated cells are intraperitoneal, yet this site is not practical as inflammation and immune responses are associated with intraperitoneal sites. Apart from intraperitoneal sites, kidney subcapsular, subcutaneous, and under the skin spaces have also been tested and have shown improved biocompatibility of encapsulated islets and a reduced macrophage recruitment. These sites are being further tested for encapsulation of islet cells to improve survival, engraftment, and function.



*Figure 6: Encapsulation devices sites: supply of nutrients and oxygen.*

Current issues involving sites that are more newly explored include death of cells by hypoxia, a condition of low oxygen levels in cells. Impaired cell growth and response due to hypoxia results in instability and lack of function through other side effects like hyperglycemia and elevated levels of fatty acid<sup>33</sup>. Other detrimental effects that must still be researched include the overgrowth of capsules in the macroencapsulation and microencapsulation devices, since they may inhibit the efficient transport of nutrients to islets, further causing hypoxia as well as necrosis, the death of body tissue due to too little blood flow. This may cause delayed

vascularization, or the improvement of nutrient supply, and ultimately lead to the implanted cell destruction and graft failure, which is characterized by the complications of allogeneic HSCT or the loss of donor cells.

<i>Table 1: Macroencapsulation vs. Microencapsulation</i>		
	Macroencapsulation	Microencapsulation
Definition	<p>System where <math>10^3</math>- <math>10^6</math> islets are enclosed in a device that contains a semipermeable barrier.</p> <p>In the context of T1D, acts as a bioartificial pancreas, immunoprotection encapsulated beta cells</p>	<p>Made of thin polymer films which tightly adhere to individual islet or cell clusters.</p> <p>Bioengineering technique capable of creating an immune-privileged site</p>
Benefits	<p>Allows the free diffusion of oxygen, nutrients, insulin, and glucose to and from the encapsulated cells.</p> <p>Evades the immune response while simultaneously allowing delivery of insulin from transplanted cells</p>	<p>Occupies a very limited graft volume and could be eligible for alternative graft sites</p>
Encapsulation Sites	<p>Most implanted within the peritoneal cavity and subcutaneous space → hypoxic condition &amp; often less vascularized</p> <p>In order to provide optimum nutrient distribution, density of the device should be 5-10% of volume</p>	<p>The only site that has been explored so far is the peritoneal cavity for implantation. Many other sites are being researched and will soon be explored.</p>

Limiting factors	Contains a limited, cell-loading capacity due to reliance on diffusion. Slow glucose stimulated insulin secretion → scaling devices for human application is difficult	Efficiency of immune barrier competence, the site of implant, and the dual dependence on the final microcapsules size.  Microencapsulated devices prevent ingrowth of vessel which limits the nutrient supply to only diffusion through the semipermeable membrane
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## Discussion

<i>Table 2: Implications</i>	
Practical Implications	Theoretical Implications
Informs T1D patients/researchers of an alternative to pancreatic transplantation, which can be inaccessible due to high costs and donor shortages	Beta cell transplantation has been regarded as a plausible and efficient method of reversing type 1 diabetes.  No longer limited to ineffective transport of large/substantial amounts of oxygen as well as nutrients
Involves reversal potential of Mesenchymal stem cells due to their ability to differentiate into functional cell types  Can offer a proper control of encapsulation devices on reversal of diabetes to prevent foreign body response	Proposals have the ability to challenge current medical barriers on finding ideal transplantation sites

<p>Poor immunosuppression increases the need for more efficient strategies, such as exploring alternative transplantation sites and various encapsulation techniques.</p>	<p>Accessibility may be boosted, breaking down financial barriers in the journey to find the ideal transplantation sites.</p> <p>Selective sites can enhance the compatibility of the devices as it pertains to transplantation and regulating the body responses, and immune barriers can be strengthened</p>
<p>Encapsulation offers optimal sites that help implantation of islet cells.</p> <p>Self renewing cells in the future can provide a stable source of insulin whilst also solving donor shortages</p>	<p>For future studies, there are immune protective agents that can better stabilize body responses and provide for more efficient, alternative transplantation sites for islet cells.</p>
<p>Both macro/microencapsulation provide potential methods to prevent detrimental foreign body response.</p> <p>Studies and current proposals lead this research towards reversing T1D in a forward, productive direction.</p>	<p>Improving characteristics of transport for nutrients and oxygen through the encapsulation devices and coupling it with other current treatments can be an advanced approach to reversing diabetes</p>

As research on macro and micro encapsulation continues to develop, potential pitfalls have been examined in order to further the efficiency of such processes. In a clinical trial held by PhD James Shapiro, such pitfalls of islet transplantation specific to the foreign body response were addressed. The trial induces the growth of new blood vessels and utilizes this foreign body response to its advantage by modifying the target site. The participant must have reduced awareness of hypoglycemia or metabolic instability and be between 18-68 years old. Through an angiocatheter tube, a site is made into a viable location, and islet transplantation will occur in the device-less sentinel space and the transplant site is removed from the pocket. This is one of the first human studies, and recipients of the islet transplants were

monitored. The outcome measures are to assess implant tolerability or the rate of inflammation at the site, as well as adverse effects on the participants to assess the effectiveness of this harnessed foreign body response

Another ongoing trial from the City of Hope Medical Center aims to assess a safer method of islet transplantation for normal control of blood sugar without needing insulin shots. New onset diabetes is common due to an organ transplant because of certain medications. The treatment is set to transplant human allogeneic islet cells with varying dosage based on patients weight. T1D patients between 18-60 years will receive immunosuppression medication during treatment. The primary outcome measures of this study are to reduce Hemoglobin A1c by at least 1 point, as well as eliminate hypoglycemic events after the 1st islet transplant.

As detailed before, Mesenchymal Stromal Cells have ample potential to cure diabetes with its ability to differentiate into different cell types and for its self-renewal abilities. A clinical trial, Cellular Therapy for Type 1 Diabetes Using Mesenchymal Stem Cells, through the medical University of South Carolina is meant to determine the efficacy of metabolically active MSCs in order to treat the new onset of type 1 diabetes as well as derive its mechanism of protection. Currently ongoing, this research uses 50 participants that will receive treatment. Group A will receive a single MSC infusion, and group B will receive a single infusion of placebo. The primary outcome measure is a 12 month change in C-peptide area under the curve and change in cell beta function. Since MSCs are effective and suppress autoimmunity, the study aims to see MSC's effect on insulin secretion rate, change in islet autoantibodies, change in beta cell death measurements, change in T-cell response, etc<sup>34</sup>.

Finally, with the introduction of a coupling method between islet transplantation and encapsulation devices, studies detailing the islet cell transplantation process prove to be useful in assessing complications that may arise. In an ongoing clinical trial on Improving Islet Transplantation Outcomes With Gastrin, sponsored by the City of Hope Medical Center, the effectiveness of Gastrin treatment with islet transplantation was evaluated. Due to the limited supply of donor islets, the study seeks to test if

gastrin, which is a natural gut hormone present in the pancreas in the embryo and a helper of the formation of the pancreas, can be injected to make fewer number of transplanted islets work in a similarly efficient manner. For this study, participants with frequent hypoglycemic episodes receive treatment with an islet transplant. For the next 30 days, they will be injected with gastrin and continue the process, as well as take anti-rejection medication, which is crucial because foreign body response as talked about before can hinder the effects of the study.

## **Conclusion**

Type 1 diabetes may be one of the most heavily researched autoimmune diseases; however, the current research into the application and potential of stem cells in pancreatic islet cell transplantation is nominal. The popular, modern-day solutions of relying on CGMs, strict lifestyle routines, and daily insulin injections are time consuming and outdated in comparison to the potential of highly effective cell research. Stem cells can differentiate to behave just like pancreatic islets and beta cells and can therefore produce insulin, and this would eliminate the need for daily insulin injections. In fact, stem cell implantation has proven to be one of the leading pathways in transplantation, with current therapies including Mesenchymal stem cells, human pluripotent cells, and even embryonic stem cells. However, implantation can prove to have more obstacles than desirable such as cost, limited donors, immunosuppression, and further unwanted foreign body immune responses that may lead to death of the transplanted cells.

Implantation of stem cells alone is risky as the chances of a host immune response is very likely, encapsulation techniques present a solution. Encapsulation methods protect the encapsulated cells from an immune response by the host, which would allow for continuous exchange of nutrients and insulin and increase the life expectancy of these transplanted cells as well. Clinical trials in humans have yet to be conducted to explore encapsulation methods, but this solution has proven to be viable in NOD mice and has the ability to provide reversal treatment for T1D. Details surrounding the most efficient encapsulation techniques and encapsulation sites are unclear, but it is found that macroencapsulation and microencapsulation provide viable solutions despite their many limitations.

To further stem cell research, a coupling of such macroencapsulation and microencapsulation devices may prove to be beneficial in the long run when facing technical issues by one device. A strategic employment of combined alternatives such as the devices to embryonic stem cell therapy can be a step towards reducing foreign body immune response as well as offering immunoprotective strategies and increasing healthy cell proliferation and nutrient exchange. The clinical trials mentioned before are currently in progress to test out such theories and identify the collective benefit of using such strategies to reverse diabetes through noninvasive treatment.

According to the current state of research surrounding stem cell therapy, the applicability of real-world stem-cell based islet transplantation is ambiguous. Once further development in research is made, a conclusion based on the biological, economical, and hazard-based results can be established. The implications of the practical progress involving developing strategies for better immunosuppression techniques and alternatives to current cell transplantation methods set the foundation for diabetic reversal. Similarly, theoretical implications including greater accessibility and alternative transplantation sites provide a reliable pathway into the future of not just diabetic reversal but for numerous autoimmune diseases. Once that future research is advanced, stem-cell encapsulation may just be the key to clinical cell therapeutics in the efficient and safe reversal of type 1 diabetes.

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