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REVIEW ARTICLE

Encapsulated Islet Transplantation: Strategies and Clinical Trials

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Encapsulation of tissue has been an area of intense research with a myriad number of therapeutic applications as diverse as cancer, tissue regeneration, and diabetes. In the case of diabetes, transplantation of pancreatic islets of Langerhans containing insulin-producing beta cells has shown promise toward a cure. However, anti-rejection therapy that is needed to sustain the transplanted tissue has numerous adverse effects, and the islets might still be damaged by immune processes. Furthermore, the profound scarcity of healthy human donor organs restricts the availability of islets for transplant. Islet encapsulation allows the protection of this tissue without the use of toxic medications, while also expanding the donor pool to include animal sources. Before the widespread application of this therapy, there are still issues that need to be resolved. There are many materials that can be used, differing shapes and sizes of capsules, and varied sources of islets to name a few variables that need to be considered. In this review, the current options for capsule generation, past animal and human studies, and future directions in this area of research are discussed.
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INTRODUCTION

There are a number of strategies to protect transplanted tissue from the host immune system. Currently, the mainstay of clinical therapy is the use of anti-rejection medications to achieve chronic immunosuppression. These agents, while having proven efficacy in extending the longevity of transplanted tissue, have numerous adverse effects (1). These range from diarrhea to cancers, and in general are extremely toxic to the host. One alternative strategy for protecting certain types of transplanted tissue is encapsulation. The goal of encapsulation is to have a permselective barrier that allows nutrients in, tissue products out, while preventing the influx of products of the immune system. Essentially, the goal is to recreate the natural barriers of the body (such as the blood-brain barrier or the blood-testes barrier), which largely serve the same purpose as encapsulation (2).

Encapsulation strategies vary, and are currently being investigated for uses as diverse from cancer therapy to neural tissue regeneration (3,4). One prominent field of study is pancreatic islet transplantation for diabetes. In this case, encapsulation is uniquely suited to benefit this effort for several reasons. Unlike other solid organ transplants, such as the kidney, heart, liver, or whole pancreas, the purpose of islet transplantation is much simpler: release the appropriate amount of one protein (insulin) in response to one signal (glucose). This makes the design goals clear - allow the flux of these two entities as well as waste and nutrients. Further discussion here will give an overview of strategies for capsule generation, data from animal trials that have laid the foundation for human trials, as well as future improvements on this technology.

CAPSULE CONSIDERATIONS

The ideal capsule will only need to be implanted once in a patient's lifetime, provide stable, predictable, and reprodu-

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Abbreviations: NHP, non human primates; T1Dm, Type 1 diabetes mellitus; IEQ, islet equivalent

cible function, and not burden the patient with immune suppressive regimens, discomfort, or other adverse effects. One of the first issues to be resolved with encapsulation are which materials should be used. There are agents found in nature, such as collagen, gelatin, hyaluronate, fibrin, alginate, agarose, and chitosan (5-7), as well as synthetic products such as poly acrylic acid, polyethylene oxide, poly vinyl alcohol, polyphosphazene, and Teflon (PTFE). Of these agents, alginate has been one of the most popular. It is bioneutral, evoking little response from the host immune system, it is resistant to oxidative damage, and forms a permselective barrier (8). However, there are detriments to alginate use, such as the frequent use of polycationic polymers (e.g. polylysine) to stabilize alginate gels, which has been shown to decrease graft function *in vivo* (9).

Apart from materials used, there are dimensions of the capsule that need to be considered. Encapsulation devices range from 'microscale' devices like alginate microcapsules to the 'macroscale' PTFE Encaptra macrocapsule. Microcapsules are small cell-containing droplets ranging from 100 nm to 1 mm in size (10,11), while macrocapsules can be as large as 3 cm by 8 cm, and hold up to 250 μl of tissue (12). They both have their advantages; microcapsules, by virtue of their size, have a shorter diffusion distance for oxygen and other nutrients, while macrocapsules have rough surfaces that can promote neovascularization (13,14). While it would be ideal to place capsules in the blood stream to optimize oxygen and nutrient delivery as well as waste removal, intravascular devices have been largely abandoned due to risks of thrombosis and hemorrhage (15). Overall, the choice of capsule size, material, and implant site must be balanced to obtain the previously stated goals of transplantation.

ANIMAL STUDIES

After initial *in vitro* studies demonstrated that encapsulated islets remained viable and functional, numerous animal studies followed. One of the first groups to perform *in vivo* studies allotransplanted alginate microencapsulated rat islets into the intraperitoneal (IP) space and compared their survival rates to that of unencapsulated islets placed in the kidney capsule. They found that post-transplant, the encapsulated islets survived up to three weeks, versus eight days for unencapsulated islets without immunosuppression (16). Following this initial experiment, numerous islet transplant studies have been performed in rodents. An article on this subject reviewing studies performed between 2000 and 2010 found 56 publications, with 19 involving islet encapsulation. Of the articles compared in that review, the best reported survival was 100 days for encapsulated islets transplanted IP, while unencapsulated islets transplanted into the liver were only able to survive 164 days despite concomitant immunosuppresion (17). Different methods for encapsulation have also been evaluated in rodents. In one macroencapsulation study, islet allografts were transplanted into the epididymal fat pad of streptozotocin induced diabetic mice. Varying concentrations of islets were mixed in an alginate solution and loaded into a device that was implanted. Four weeks post-transplant, mice attained normoglycemia lasting up to 12 weeks. The optimal loading density of islets used was 500 per device, with no additional benefit seen at higher numbers. It was observed that in the mice where this therapy failed there was a noted increase in fibrosis around the macrocapsule (18). Studies have also investigated biomaterial choice *in vivo*. In one study, it was noted that high glucoronic acid alginate decreased capsule retrieval at explant, poly-l-lysine coated capsules were more susceptible to breaking and produced a stronger fibrotic reaction, and cross linkage with $BaCl_2$ resulted in reduced fibrosis when compared with $CaCl_2$ (19). These numerous studies performed on rodent models have provided us with critical information regarding islet dose, choice of biomaterials, and the expected course of transplants *in vivo*, as well as lay the foundation for later large animal studies.

Large animal studies have been conducted in various species, from dogs to non-human primates (NHP). In one canine study microencapsulated islets were transplanted IP, and using C-peptide analysis survival of grafts was found to be up to 726 days (20). This long term survival was attributed to careful selection of high quality capsules. In showing the importance of capsule selection, a study was done where porcine islets transplanted into NHP were either unencapsulated, poorly encapsulated, or quality encapsulated. Poorly encapsulated islets were rapidly rejected at a rate similar to unencapsulated islets, while quality islets lasted six months (21). Like previous rodent studies, macro versus microencapsulation has been studied in large animals. In a comparison of porcine islets placed within a dual-layer alginate macrocapsule transplanted subcutaneously versus alginate microcapsules placed under the kidney capsule in NHP, macrocapsules fared better than microcapsules. Macrocapsules provided normoglycemia for up to 6 months, as compared to

two weeks for microcapsules (22). In another NHP study, microcapsules were placed IP at a dose of 10,000 IEQ/kg, with exogenous insulin requirement decreasing to 36% at 12 weeks, and 43% at 23 weeks compared to controls (23). Due to these promising results in large animal studies demonstrating that encapsulated islet transplant decreased the burden of diabetes without the use of immunosuppression, researched progressed to clinical trials.

CLINICAL TRIALS

The first human clinical trial in encapsulated islet allotransplantation was performed in a 38 year old type 1 diabetic (T1DM) male. Cadaveric human islets were encapsulated in alginate microcapsules, and placed IP at a dose of 10,000 IEQ/kg, with a 5,000 IEQ/kg booster given six months later. The patient was able to discontinue all exogenous insulin at nine months. However, it is important to note that the patient was already on anti-rejection medications due to a renal transplant (24). This initial success though did demonstrate that encapsulated islets were able to achieve glycemic control in a T1DM patient similar to unencapsulated islets placed in the portal vein. A following study done in four T1DM patients transplanted with microencapsulated islets provided a significant reduction in exogenous insulin requirements for a period; however at a seven year follow-up all patients were back to their pre-transplant doses of insulin (25). Similar results were obtained using alginate microcapsules in two patients in a separate trial, with both patients reducing their exogenous insulin requirements, but never attaining complete insulin independence (26). In taking a look at why therapy eventually tapers off in human trials, a trial had one subject undergo laparoscopy to inspect the islets. This patient still had detectable c-peptide, but not enough insulin to adequately control his diabetes. The capsules were found to be surrounded by fibrous tissue, and in some cases the islets were necrotic (10). These results mirror those from the previously mentioned rodent study, where increased fibrous tissue deposition negatively impacts encapsulated islet function.

Unlike the aforementioned human trials that used alginate microcapsules, one study evaluated macroencapsulation in patients. Twelve T1DM patients received porcine islets at a dose of 10,000～20,000 IEQ/kg placed in a collagen matrix in stainless steel mesh tubes, with a PTFE rod in the cassette as well. These devices were placed subcutaneously in the anterior abdominal wall. The purpose of the PTFE rod was to induce neovascularization, and it was removed two weeks post implant. In eleven of these initial patients a second and third device were placed six to nine months later. Six patients had a significant decrease in exogenous insulin requirements for up to four years, with two of these six becoming insulin independent for several months. In four patients, the macrocapsules were removed and beta cells were detected (25). In another trial using this macroencapsulation method in 23 patients, 11 patients had a 33% reduction of insulin requirements (26). Apart from exogenous insulin requirements, studies have also monitored other parameters as an index of adequate glycemic control in islet transplant patients. In one group transplanted with alginate microencapsulated porcine islets a significant decrease in hypoglycemic episodes, attributed to a decrease in unaware hypoglycemia (20 incidents pre-transplant, 8 incidents post-transplant after 12 weeks), was noted regardless of the islet dose used (27). In a single-patient report, researchers found that HbA1c decreased from 9.3 to 7.8 fourteen months after receiving the transplant. This patient had received microencapsulated porcine islets, which also produced detectable C-peptide for up to 11 months. The patient's insulin requirements unfortunately returned to his pre-transplant levels at 49 weeks post-transplant. Of note in all the previously mentioned clinical trials of porcine tissue pathogens such as porcine endogenous retrovirus did not cause any infection in human hosts (11). Overall, the use of encapsulated porcine islet xenograft has benefited patients by temporarily reducing the burden of diabetes without the need for toxic anti-rejection therapy. However, therapy eventually fails, and active investigation is currently underway to improve existing technology to achieve the goal of a permanent cure.

FUTURE IMPROVEMENTS

As noted in previously mentioned studies, a fibrotic reaction to the graft correlates with poor islet function and failure of therapy (10,18,19). Even in alginate microcapsules that are designed to be bioinvisible, fibrosis still occurs. Whether or not the actual process of fibrosis is the culprit for failure, the inflammatory process at large involves numerous cytokines. Of note are interleukin-1 (IL-1) and tumor necrosis factor alpha (TNF-α) that have been shown to cause graft injury and failure (13,28,29). To combat these effects, one group experimented with an IL-1 inhibitor *in vitro* with encapsulated islets exposed to proinflammatory cytokines. Islets treated

with the inhibitor were 60% more viable than controls (30). Another study used steroids (dexamethasone) to decrease the inflammatory reaction to the transplant process. Capsules placed IP with dexamethasone had less fibrosis than those transplanted without (31).

In addition to fibrosis and the inflammatory process, there is a need to optimize oxygen and nutrient delivery to encapsulated islets. As mentioned earlier, intravascular implantation has been associated with prohibitive thrombotic and bleeding risks; therefore, we require a different method to optimize circulation to islets (32,33). The ideal PO_2 for optimal islet function is 60 mmHg, with islet function dropping to half at 27 mmHg, and 2% of that at 5 mmHg (32). Even in studies with little or no fibrosis, graft necrosis in the center of the islets revealed insufficient oxygen and nutrient delivery to meet the metabolic demands of the tissue (34). One approach has been to engineer an oxygen-generating biomaterial. Solid calcium peroxide encapsulated in polydimethylsiloxane was used to deliver oxygen to the encapsulated islets for six weeks. When placed *in vitro* with encapsulated islets, this demonstrated improved insulin release in response to glucose while decreasing LDH and caspase activity (35). Other methods, such as encapsulation with vascular endothelial growth factor and fibroblast growth factors, have also been demonstrated (14,36,37). Use of growth hormone-releasing hormone-treated islets encapsulated within alginate macrocapsules resulted in a faster reversal of hyperglycemia and more consistent euglycemia as compared to untreated controls (38).

CONCLUSION

Studies performed in several different small and large animal models have demonstrated the promise of encapsulated islet xenotransplantation to treat diabetes. While currently therapy doesn't provide permanent independence from exogenous insulin, the next steps are clear. Effective methods of oxygen delivery, the use of anti-inflammatory factors, optimized biomaterial and encapsulation methods, as well as greater clinical experience with transplantation will eventually result in a solution that eliminates the need for immunosuppression in transplantation and the unreliable glycemic control achieved by exogenous insulin administration. Furthermore, gains made in islet encapsulation will lend to other encapsulation applications, resulting in exciting novel therapies for disease.

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CONFLICT OF INTEREST

The authors in this publication declare no conflict of interest.

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