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Encapsulated cell grafts to treat cellular deficiencies and dysfunction

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I. INTRODUCTION

The pathologies of several diseases are attributable to the absence or malfunctioning of specialized cells – for example, the loss of dopaminergic neurons in Parkinson’s disease or of beta cells of the pancreatic islets in diabetes mellitus (islets are cellular clusters that contain alpha and delta cells in addition to the insulin-producing beta cells). Exogenous drugs are often administered to restore normal function – for instance, in people with diabetes, exogenously administered insulin can substitute insulin produced by normally functioning beta cells. However, the periodic infusion of drugs does not mimic the complexities and subtleties afforded by the cellular regulation of biological processes, which can result in devastating consequences. Therefore, organ transplantation has been used to restore complex biological function and to provide an endogenous and inexhaustible source of therapy, thereby eliminating the need for continual access to drugs. Even so, in many cases the utility of organ transplantation is restricted by the shortage of donor human organs, the risk associated with transplant surgery and the deleterious effects of lifelong post-transplantation pharmacological immunosuppression that is used to prevent graft rejection by the host immune system.

The infusion of normally functioning donor cells can also be used to restore or retain physiological function. Furthermore, physiological function can be altered or enhanced by transplanting cells that have been engineered to perform specific tasks; cell transplantation can be used for the *de novo* production and delivery of biotherapeutic molecules. As is the case with organ transplantation, cell transplantation has the potential for lifelong therapy, typically with relatively minor surgery and fewer associated risks and complications. A variety of diseases or chronic conditions such as diabetes, Alzheimer’s disease, cancer, liver failure, anemia and chronic pain can be addressed with such cell therapy, as detailed in Table 1.

II. CELL THERAPY: SOURCES OF THERAPEUTIC CELLS

In this section, we categorize cell therapy as the transplantation of (1) differentiated, specialized native cells derived from a donor, (2) genetically modified cells that have been engineered to enhance, suppress or otherwise alter host function and (3) stem cells, which are undifferentiated and can therefore be guided by microenvironmental cues to differentiate into cells that perform highly specialized tasks.

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II.A. Native Cells

Native functioning cells may be of autologous origin (i.e. cells that have been sourced from the individual who will receive the transplant, the host) or they may be allogenic (i.e. sourced from a donor belonging to the same species) or xenogenic (i.e. sourced from a donor of a different species).

1. Autotransplantation—Autotransplantation involves the harvesting of differentiated tissue-specific cells from the host. These harvested cells are cultured *ex vivo* and reintroduced into the host to restore functional cell mass and normal activity. The procedure may be used to expand the number of functioning cells and/or to reintroduce them at particular sites of damage as is the case in the successful clinical repair of damaged cartilage using chondrocytes,¹ or to treat burns and wounds using keratinocytes.² The procedure may also be used to recover and reintroduce healthy cells upon the retrieval of damaged organs. For instance, if a tumor-bearing organ is removed, normally functioning cells from the unaffected part of the organ can be harvested and transplanted to restore host function.

Autologous cell transplantation is not susceptible to rejection by the host's immune system except in the case of autoimmune diseases, and it is suitable for tissues that can withstand surgical resection and *ex vivo* culturing. It is possible for autografts to fail owing to inflammation and the *ex vivo* expansion of adult cells is not assured.³

2. Allotransplantation—Allotransplantation involves the isolation of cells from donors of the same species, typically from cadavers. Allogenic cells have been grafted for skin and cartilage replacement^{4,5} as well as to reverse diabetes.⁶ However, allografts are subject to immune rejection by the host, where the interaction between the graft and the host's T cells results in the activation of cellular immunity. Therefore, clinical allotransplantation is accompanied by the administration of immune-suppressive drugs. These drugs can be harmful to both graft and host and increase the risk of infection and cancer.^{7,8} The clinical utility of allotransplantation may be enhanced by less harmful immunosuppressants or by rendering the allografts immune resistant through genetic modification.³

3. Xenotransplantation—Xenotransplantation involves the grafting of cells that are sourced from another species, thereby allowing for the large scale production of cells in culture and in non-human hosts for transplantation in humans. Cells may be sourced from animals that have been genetically modified to upregulate the production of therapeutic molecules, which in turn can reduce the transplantation load that is necessary for therapy. Xenografts are in clinical trials to treat diabetes, hepatic failure and chronic pain, and their application to treat several other diseases has been explored.⁹ Xenografts are subject to immune rejection through humoral immunity, which is mediated by secreted antibodies and through complement activation, necessitating the use of immunosuppressive drugs with attendant problems as stated above. The risk of cross-species infection (i.e. xenozoonosis) is a primary concern with xenotransplantation.^{10,11}

II.B. Genetically Modified Cells

Cellular activities including cell proliferation, differentiation, regeneration and secretion are primarily regulated and coordinated by gene and protein expression. Therefore, the regeneration of cells tissues and organs can be controlled by modulating gene and protein expression through the transfer of genetic material to cells to amplify or suppress factors influencing health and disease. Such genetic engineering can also impart immune resistance to cells or enable them to produce and deliver biotherapeutic molecules.¹² Furthermore, using gene promoters, it is possible to program these cells to upregulate or downregulate the factors of interest based on physiological needs and cellular milieu.

To achieve the aforementioned control over cellular activity in the host, explanted cells can be genetically modified and propagated prior to transplantation. Cells may be propagated in culture or in transgenic animals; *in situ* modification of cells *in vivo* is also possible. Examples of genetically modified cellular grafts for therapy include fibroblasts secreting erythropoietin for treatment of anemia,¹³ myoblasts secreting adenosine in the treatment of epilepsy¹⁴ and cells engineered to express tumor inhibitors or antiangiogenic molecules for cancer therapy.¹⁵ The advantage of this approach is that it does not require modification of the host genome to achieve the desired outcome.

II.C. Stem Cells

Stem cells are characterized by their capacity for self renewal and their ability to differentiate into specific cell types under the influence of their microenvironment. For example, human neuronal stem cells differentiate into muscle cells when implanted into skeletal muscle,¹⁶ bone marrow cells differentiate into neuronal cells when transplanted into neural tissue,^{17,18} liver cells transdifferentiate into islet-like cells.¹⁹ As an autologous source, stem cells offer the potential advantage of overcoming immune rejection. However, this may not be case for autoimmune diseases where the transplanted stem cells can be subjected to the same immune mediated destruction as the native cells. A major advantage of stem cells is that they can be expanded *ex vivo* and transplanted. *Ex vivo* differentiation into specific cell types, or genetic modification to impart desired characteristics, enhances the therapeutic potential of stem cells. For example, mesenchymal stem cells, which are known to be hypoinmunogenic, can be genetically modified to express therapeutic molecules while retaining their native hypoinmunogenicity.²⁰

Though, stem cells provide a potential unlimited source of cells for transplantation, their clinical application is restricted primarily owing to the limited understanding of stem cell biology. Their uncontrolled growth and proliferation or unguided differentiation leads to the formation of teratomas and teratocarcinomas.^{21,22} Encapsulation of stem cells provides a physical barrier between graft and host and can inhibit the formation of teratomas, providing a platform for clinical application.^{23,24}

III. IMMUNOREJECTION OF CELLULAR GRAFTS

Here, we briefly introduce the reader to the principal mechanisms of graft immunorejection and to strategies for overcoming this rejection (Fig. 1). In the following section, we focus on one particular strategy and its attendant rationale – the strategy of encapsulating the cellular grafts within selectively-permeable barriers to protect them from immunorejection.

III.A. Graft Immunorejection

The immune rejection of cellular grafts is mediated through a variety of mechanisms. The immediate rejection of cellular grafts is primarily attributable to hyperacute rejection (HAR) where host antibodies target antigens on the surfaces of the grafted cells. The cell membrane saccharide α -Gal is the antigen primarily implicated in HAR. Present in most mammals, α -Gal is absent in human cells. The removal of α -Gal from cells prior to grafting can eliminate HAR. Encapsulation of cells in immunoprotective barriers can also eliminate HAR by preventing cell-host contact.²⁵ Instant blood mediated inflammation reaction (IBMIR) is another mechanism of graft rejection. IBMIR is characterized by platelet consumption, complement activation and the initiation of the coagulation cascade.²⁵ IBMIR mediated graft rejection has been overcome by using anti-coagulation agents such as heparin or low molecular weight dextran sulfate.^{26,25} IBMIR may be suppressed by genetically modifying cell grafts prior to transplantation.^{27,25} IBMIR can also be overcome by transplanting grafts

characterized by poor vasculature.²⁸ Grafts are also susceptible to rejection mediated by T cells and macrophages.²⁹

III.B. Strategies to Overcome Immunorejection

Strategies to overcome graft immunorejection include 1) pharmacological suppression of the host immune system, 2) tolerance induced in the host immune system, 3) accommodation, where changes in the graft protect against rejection, and 4) encapsulation of the graft to prevent harmful cells (e.g. macrophages) and molecules (e.g. antibodies) of the host immune system from physically accessing the graft.³⁰ The first two strategies rely on modulating native host immunity, thereby leaving the host vulnerable to infection.^{31,32} Accommodation strategies involve modification of graft surfaces by treatment with molecules like heparin sulfate or through pre-transplant desensitization of the grafts by controlled exposure to cytotoxic elements.^{33,34} Encapsulation relies on a physical barrier between the graft and host for immunoprotection and therefore may be most suitable for host health and graft function.

IV. IMMUNOPROTECTIVE ENCAPSULATION OF CELLULAR GRAFTS

The principal aim of graft encapsulation is to isolate the graft from the host using a selectively permeable physical barrier between the two, a concept that was introduced almost half a century ago.³⁵ The barrier permeability is selected to allow the bi-directional diffusion of small molecules between the graft and the host. These small molecules include oxygen, carbon dioxide, cellular nutrients and growth factors, cellular waste products, ions, and therapeutic molecules secreted by the grafted cells. The permeability is also selected to prevent the transport of cells, such as macrophages, and large molecules of the host immune system, such as antibodies and complements, which can damage the graft.

The choice of encapsulating material is determined by the nature of application. Biodegradable polymers may be advantageous in short-term applications, such as for the oral delivery of genetically engineered *E. coli* to remove urea,³⁶ whereas long term applications, such as the treatment of chronic diseases, require mechanical stability and long-term biocompatibility. The therapeutic application of encapsulated cells has been demonstrated in a variety of disease conditions emphasizing the diversity of the technique as a potential treatment modality (see Table 1).

IV.A. Microencapsulation

Microcapsules are characterized by dimensions of the order of hundreds of microns or less. The small encapsulation volume enables capsule implantation in microvasculature, deep tissue and difficult to access sites. The high surface area-to-volume ratio of microcapsules enables efficient diffusion of small molecules.

1. Alginate Encapsulation—Typically, graft encapsulation is achieved through the entrapment of cells in spherical microbeads that are made of a selectively permeable polymer gel matrix (Fig. 2). Sodium alginate is the most common polymer matrix used to generate cell-encapsulating microbeads. Alginate is a marine polysaccharide comprising guluronic acid and mannuronic acid. Guluronic acid residues are exposed to the surface and impart a net negative charge to the polymer, which ionotrophically gels on contact with [positively charged] calcium ions. Typically, cells are suspended in an alginate solution which is extruded through a droplet generator. The droplets fall in a calcium chloride bath, leading to the formation of microbeads with cells entrapped in them. Alginate has been successful in encapsulated cell therapy for short and intermediate term application.³⁷

Key challenges in the use of alginate for cell encapsulation include a) its immunogenicity, b) its lack of chemical and mechanical stability, and c) the lack of precise control over its porosity. Commercially available alginate is sterilized and treated with antimicrobial compounds to negate mitogenic and cytotoxic contaminants. However, they still contain residual proteins that induce inflammation and fibrotic overgrowth with adverse consequences for long term graft survival.³⁷ The polycation poly-L-lysine (PLL) has been layered on alginate surfaces to improve the gel's mechanical stability and to provide better control over microbead permeability by varying the PLL concentration and its reaction time with alginate.³⁸ Yet, alginates with a low concentration of guluronic acid swell and lead to fracture of the PLL layer releasing soluble PLL molecules which can induce inflammation and lead to graft failure.³⁹ More recently, the use of alternatives to PLL such as poly-L-ornithine (PLO)⁴⁰ and poly (methylene-co-guanide)⁴¹ has improved microbead stability and concomitantly resulted in enhanced microbead biocompatibility. Multilayered polyelectrolyte coatings provide improved control over gel porosity and therefore enhance the immunoprotection efficiency of alginate.^{42,43} Barium cross-linking of alginate has also been reported to increase the alginate's mechanical stability. However, long gelation times which are essential for the generation of uniformly cross-linked barium-alginate spheres are not feasible considering the toxicity of barium ions. A crystal gun method which injects a jet of nanocrystalline barium ions into the alginate droplets before they reach the gelling bath enhances the homogeneity of barium-alginate beads and mitigates toxicity⁴⁴

In spite of the aforementioned efforts to address the key challenges associated with alginate encapsulation, there are fundamental drawbacks in its use for long term cell encapsulation application such as cell therapy for chronic diseases. Alginate scavenges calcium and magnesium, thereby inhibiting ionic processes. Calcium in the alginate matrix can be partially replaced with sodium, which results in microbead swelling and a loss of its mechanical strength.⁴⁵ The lack of precise nanometer-scale control over alginate porosity results in insufficient graft immunoprotection. Alginate microbeads are large and the large distances between the microbead surface and the entrapped cells result in delayed graft response to secretagogues.⁴⁶

2. Conformal Coating of Cells—A very thin immunoprotective coating on grafted cells or cell aggregates can reduce the molecular transit time between graft and host, thereby enabling rapid graft response to changing host needs. The thin coating also drastically reduces the volume of the transplant, i.e. transplant load. Sefton *et al.*⁴⁷ conformally coated cell aggregates within a thin layer of hydroxyethyl methacrylate-methyl methacrylate (HEMA-MMA) employing a low speed centrifugation process through a gradient of alternate aqueous and organic layers. The beads were initially coated with a layer of HEMA-MMA polymer, excess polymer was washed away and the coated cell aggregate was thinly and conformally coated. Wyman *et al.*⁴⁸ conformally coated pancreatic islets based on the selective withdrawal of islets from an oil-water bilayer. Islets were suspended in an oil-water bilayer using a chlorinated hydrocarbon oil which is denser than water. Islets being heavier than water but lighter than oil float at the interface and when oil was drawn from just below the interface, islets were sucked into the capillary entrained in a thin layer of oil and suspended in the aqueous layer. The aqueous layer comprised of photopolymerizable PEG-diacrylate mixed with eosin as a photoinitiator. Subsequent, photoinitiated crosslinking resulted in the generation of islets with a uniformly coated PEG layer, approximately 50µm thick (Fig. 3).

A layer-by-layer (LbL) coating of polyelectrolytes, using alternate layers of polycations and polyanions results in precise control over the thickness of the coating. The choice of polymers, their concentration and the number of layers can be modulated to control coating thickness in the nanometer range. LbL can be used to generate thin films on non-planar

substrates. Examples of commonly used polyanions are alginate, polystyrenesulfonate (PSS) and poly(acrylic acid) (PAA) while that of polycations are polyallylamine hydrochloride (PAA), poly(diallyldimethylammonium chloride) (PDMA) and poly-L-lysine (PLL). Multilayered polyelectrolytes⁴² and PEG hydrogels have demonstrated more facile control over matrix porosity.⁴³

In addition to the aforementioned alginate and polyelectrolyte matrices, a variety of natural and synthetic polymers have been evaluated for cell encapsulation to improve control over porosity and to enhance biocompatibility.^{49–51}

3. Encapsulation in Nano- and Micro-fabricated Devices—Despite tremendous progress in developing hydrogels and polyelectrolytes for encapsulation, the lack of strictly controlled, monodisperse porosity prevents their use in applications requiring long term and complete graft immunoprotection. The failure of even a small number of pores to meet critical dimensions can result in antibody passage and immunorejection. For applications that demand well controlled, uniform and reproducible porosity of the graft encapsulating membrane, inorganic membranes can be fabricated using techniques typically used in the semiconductor industry. These membranes, on the surfaces of graft-encapsulating devices, can provide complete and permanent pore-size exclusion of molecules.

Chu *et al.*⁵² fabricated nanoporous membranes with 10 nm pore size using polysilicon and silicon dioxide. The membrane filters comprised lateral nanoslots which efficiently block large immune molecules. However, the membranes also inhibited the free diffusion of small molecules of interest. Desai *et al.*^{53,54} micromachined nanoporous membranes from silicon oxide, with pore diameters of about 18 nm and demonstrated the retarded diffusion of IgG molecules through small pores. Alumina membranes with pore diameters of the order of 25 – 55 nm were fabricated through a process of anodization of alumina and subsequent etching of the formed oxide layer.⁵⁵ Gimi and colleagues^{56,57} have created nanoporous membranes in an epoxy-based polymer using electron-beam lithography and nanoimprint lithography. The latter technique has the advantage of extremely high-throughput fabrication of nanochannels whose cross-sectional dimensions can be precisely controlled and whose depths can be arbitrarily defined (Fig. 4).

Absolute exclusion of cytotoxic molecules cannot be achieved as they span a broad range of molecular sizes, including molecules comparable in dimensions to essential nutrients. The dynamic structural conformation of proteins can enable their diffusion through pores smaller than their expected hydrodynamic radii.⁵⁸ Additionally, shed antigens from the encapsulated cells, which are implicated in the greater failure of encapsulated xenografts in comparison to allografts, can also leak out of the encapsulation space and elicit host immune response. Reducing the pore diameters to exclude shed antigens is not possible since it also precludes the diffusion of essential small molecules.⁵⁹ Iwata *et al.*⁶⁰ had observed that absolute exclusion of complements is not essential as they are deactivated during the process of diffusion through the nanoporous channel. Hence, in addition to pore diameter, the depth and geometry of the nanopores is also of critical importance as longer diffusion lengths can inactivate complement molecules.

IV.B. Macroencapsulation

Macrocapsules are characterized by dimensions of the order of 0.5–1.5 mm in diameter and a few cm in length. The large encapsulation volume of macrocapsules facilitates high cell loading densities, which reduces the number of capsules needed for implantation. Implantable macrocapsules have been developed as bioartificial pancreata.^{61–63} The typically thicker membrane walls of these macrocapsules provide enhanced mechanical stability but at the same time hinder diffusion of essential nutrients. The problem of

diffusion is circumvented in arteriovenous (AV) shunts, which are essentially macrocapsules immobilized on the vasculature enabling circulation through their hollow core. Cells are immobilized on the surface of the AV shunts and are thus in close contact with the circulating blood, enhancing the response characteristics. A major advantage of macrocapsules is the ease of retrieval in case of graft failure. The use of macrocapsules is limited by the acute inflammatory response elicited in host tissue due to the large size of macrocapsules.^{64,65} Despite the limitations, successful implantation of AV shunts comprising a single coiled membrane with immobilized pancreatic islets was demonstrated in dogs.^{66,67} Long term biocompatibility of cylindrical diffusion devices made of polyacrylonitrile-polyvinylchloride (PAN-PVC)⁶⁸ led to the development of islet implants which were shown to reverse hyperglycemia in dogs and rodents over several months.⁶⁹

V. APPLICATIONS AND CLINICAL TRIALS

In this section, we discuss encapsulated cell therapy for two diseases – type 1 diabetes and cancer – and related clinical trials. Many candidate diseases for encapsulated cell therapy result from hormone deficiencies and can therefore be managed by endogenous hormone replacement using encapsulated cells. For that reason, we first focus on type 1 diabetes which is characterized by the deficiency of the hormone insulin, and because it is the most extensively studied disease for encapsulated cell therapy. Then we focus on encapsulated cell therapy for cancer to highlight the multi-factorial and versatile nature of this therapy. Cancer is a complex and protean disease with multiple factors implicated in disease onset, progression, aggression and remedy. Encapsulated cells can be used to directly target cancer cells (e.g. with cytotoxic agents) or they can be used to target the tumor microenvironment (e.g. choking-off tumor vasculature to starve the cancer cells). Several factors/pathways may be targeted in tandem to improve therapeutic outcomes. Furthermore, the expression of therapeutic proteins can be modulated using tissue-specific or environment-specific cues to adapt to changing pathology. Therefore, the encapsulation of an engineered cell line that can perform multiple tasks, or the co-encapsulation of several specialized cell types, can serve as a combinatorial approach in curing or managing complex diseases.

While we focus on diabetes and cancer as explained above, the clinical utility of encapsulated cells has been explored in the therapy of other diseases such as retinal degeneration; clinical trials using encapsulated engineered cells designed to produce ciliary neurotrophic factor to reverse the effects of retinal degeneration are in progress.^{70,71}

V.A. Type 1 Diabetes Therapy

As indicated earlier, type I diabetes is an autoimmune disease that is characterized by immune mediated destruction of the insulin-producing beta cells of pancreatic islets. Though exogenous insulin administration continues to be the primary mode of therapy for type 1 diabetes, the restoration of functioning islets or beta cells through transplantation is a therapeutic alternative. The transplantation of pancreatic islets was first proposed in 1967⁷² and was demonstrated in a rodent model in 1972, by reversing chemically induced diabetes.⁷³ Recent years have witnessed considerable progress in the design of bioartificial pancreata to overcome exogenous insulin dependence.^{74–83} The transplantation of pancreatic islets is now clinically practiced in type 1 diabetes management.

1. Sources of Pancreatic Islets—Islets for transplantation are primarily sourced from human donors. However, xenogenic sources are attractive owing to limited human donor availability. While there is a vast pool of potential xenogenic islet sources, porcine islets are particularly attractive owing to the structural similarity between porcine and human insulin,⁸⁴ and because neonatal porcine islets can mature into functional endocrine tissue.⁸⁵ Andersson *et al.*⁸⁶ reported a clinical trial involving the transplantation of fetal porcine islet-

like clusters with attendant use of pharmacological immunosuppression, and more recently, alginate-encapsulated porcine islets were used for transplantation without the use of harmful immunosuppressive drugs.⁸⁷

2. Insulin Producing Cells or Surrogate Beta Cells—Since the beta cells of the islet are responsible for insulin production, they may be transplanted in lieu of intact islets. Alternately, surrogate beta cells, i.e. cells that mimic beta cell function by producing insulin, may also be used for grafting.

The multiple sources of insulin-producing cells include i) beta cell precursor cells, ii) stem cells that differentiate into functional beta cells, iii) pancreatic ductal epithelial cells that differentiate into beta cells, iv) acinar cells that transdifferentiate into beta cells, among others.^{88–90} Pharmacological interventions can accelerate some of these processes; beta cell neogenesis can be stimulated with peptides such as exendin-4,⁹¹ glucagon-like peptide 1⁹² and islet neogenesis-associated protein (INGAP) peptide.⁹³

Human and murine embryonic stem cells (ESCs) can differentiate into surrogate beta cells as a result of genetic manipulation⁹⁴ or under appropriate culture conditions.^{95,96} Early attempts at such differentiation resulted in cells with weak insulin production, poor response to glucose challenge and uncontrolled differentiation post transplantation. Subsequently, efforts to first differentiate ESCs into endodermal cells with soluble factors,^{97,98} and then differentiate them into pancreatic cells, have achieved controlled differentiation and improved function.⁹⁹ Non-embryonic stem cells can also differentiate and transdifferentiate into surrogate beta cells following the gene transfer of transcription factors such as pancreatic duodenal homeobox 1, which is vital in pancreas development. Progenitor cells may be sourced from the pancreas or from non-pancreatic origin such as hepatocytes, interstitial epithelial cells, mesenchymal stem cells and stem cells from bone marrow.^{89,100}

3. Enhancing the Stability of Beta Cells—In addition to developing alternate sources of beta cells, a variety of strategies have been employed to enhance beta cell graft survival. These approaches include i) the co-transplantation of protective cells, ii) the removal of surface antigens, iii) genetic engineering of grafts to secrete protective peptides, iv) using gene transfer to inhibit apoptosis, and v) enhancing anti-oxidant protection.⁸⁸ For instance, Calafiore and colleagues have demonstrated enhanced graft survival and function by co-encapsulating islets with protective agents with known anti-inflammatory¹⁰¹ and antioxidizing effects.¹⁰²

4. Clinical Trials for the Transplantation of Unencapsulated/Naked Islets—

Allogenic islet transplantation in humans, accompanied by pharmacological immunosuppression, was first reported in 1990. In this study, grafting resulted in insulin independence for up to 5 years.¹⁰³ Graft loss was estimated at 50% within two months and insulin independence was estimated at 10% after one year.^{104,105} The steroid-based immunosuppressive drugs impeded insulin secretion^{106,107} A subsequent clinical trial used non-steroidal immunosuppression, establishing the *Edmonton Protocol*.¹⁰⁸ While this protocol is still used for islet transplantation, graft rejection and long term survival remain a challenge, with patients requiring additional transplantations after 2–3 years.^{109,110}

5. Clinical Trials of Encapsulated Islets in Diabetes Therapy—As opposed to the transplantation of naked islets, islets encapsulated in semi-permeable matrices are putatively immunoprotected to enable the transplantation of both allo- and xeno-grafts without the need for immunosuppressive drugs. One of the first clinical trials using encapsulated allogenic islets was initiated by Calafiore *et al.*¹¹¹ in 2003 on ten patients with long-standing type 1 diabetes and on extensive insulin therapy. For each patient, islet grafts were sourced

from a single human pancreas, encapsulated in sodium alginate microcapsules, further coated with layers of poly-L-ornithine and alginate. Approximately 400,000–600,000 encapsulated islets (in 100 ml total volume) were microinjected into the peritoneal cavity through an abdominal incision. Two patients indicated reduced insulin dependence and detectable C-peptide levels in the 6–12 month window, C peptide being an established marker for insulin secretory capacity. Although no patients could be withdrawn from exogenous insulin, the trial suggests that encapsulated islets can be viable post transplantation.¹¹¹ This development warrants further investigation and the honing of techniques for encapsulated islet therapy.

More recently, a clinical trial by Tuch *et al.*¹¹² used barium alginate to encapsulate human islets for subsequent infusion in 4 patients with long standing type 1 diabetes, no detectable C-peptide levels and on exogenous insulin therapy. Islets were sourced from a human donor, encapsulated, and periodically infused into the peritoneal cavity over 19 months through an abdominal injection.¹¹³ The study reported the presence of C-peptide one day post transplantation, but the levels subsequently declined and were completely absent after 1–4 weeks. Similarly, blood glucose levels and exogenous insulin requirements decreased after the first day but subsequently increased to pre-transplant levels. However, one patient showed detectable C-peptide levels after 2.5 years, though the levels were insufficient to alter exogenous insulin requirements. An analysis of the extracted microcapsules indicated graft failure through immunorejection.¹¹²

Xenotransplantation is particularly attractive since it can potentially overcome the shortage of human donor organs and provide a vast source of islets for transplantation.¹¹⁴ One of the first examples of xenotransplantation in humans involved the grafting of neonatal porcine islets that were encapsulated for transplantation.¹¹⁵ A type 1 diabetes patient was infused with alginate-PLL microcapsules; approximately 1.5 million islet equivalents were transplanted into the peritoneal cavity. Post-transplantation dependence on exogenous insulin reduced by ~ 30% over a period of 14 months but reached pre-transplantation levels thereafter. Islets were retrieved after 9.5 years, were found to be viable, but showed very little insulin secretion. Subsequently, this work was extended to a clinical trial on 7 patients using alginate poly-L-ornithine encapsulated neonatal porcine islets without the use of immunosuppressants.¹¹⁶

Approximately 5,000–10,000 islet equivalents per kg of bodyweight were transplanted in patients with 1–3 transplants per patient; patients were simultaneously treated with insulin infusion. Two of the seven patients presented short-term insulin independence; the other patients reported reduced insulin dependence. No adverse reactions, including xenosis, were observed. Results from the study indicate the feasibility of using encapsulated porcine islets without the risk of infection. A similar study on 8 patients has been initiated this year.

V.B. Cancer therapy

Cell grafting for cancer therapy may include i) cells that secrete chemotherapeutic agents against tumor cells, or cells that express specialized enzymes that convert a non-toxic prodrug into a cytotoxic drug at the tumor site, ii) cells that secrete anti-angiogenic molecules targeted to the tumor vasculature, iii) cells that secrete cytokines that induce the host's immune system to target the tumor. In all the above applications, the grafts can be encapsulated for immunoisolation, as described earlier, and/or for sequestration at the site of interest to prevent their undesirable proliferation in the body.

1. Chemotherapy—Chemotherapy for tumor cell kill is most widely used in the clinical management of cancer. However, it is hindered by various factors such as insufficient drug concentration in tumors, high systemic toxicity and lack of selectivity for tumor cells over

normal cells. Among the numerous approaches to overcome these drawbacks, enzyme-prodrug therapy is gaining prominence since it provides a high localized concentration of the drug at the tumor site. Localizing the enzyme that mediates prodrug-to-drug conversion, or the cells that produce this enzyme, increases the drug concentration at the site of choice while concomitantly reducing systemic toxicity. Well-studied enzyme-prodrug combinations include the herpes simplex virus-thymidine kinase/ganciclovir (HSV-TK/GCV), cytochrome P450 2B1 (CYP2B1)/ifosfamide and cytosine deaminase/5-fluorocytosine,^{117–120} and carrier cells can be engineered for *in situ* expression of functional enzymes.^{121–124} The immunogenicity of such carrier cells preclude their direct administration in the body – immunoisolation is essential¹²⁵ and can be achieved through encapsulation. Encapsulated cells expressing the enzyme CY2B1, that converts the nontoxic prodrug ifosfamide into 4-hydroxyifosfamide, which then spontaneously decays to the cytotoxic molecules phosphoramidate mustard and acrolein, have been used to treat pancreatic tumors.^{126–129}

2. Anti-angiogenic Therapy—Cells expressing the antiangiogenic molecules angiostatin and endostatin have been encapsulated in alginate microcapsules and are shown to be effective in suppressing tumor growth. Baby hamster kidney cells expressing endostatin and encapsulated in alginate—poly-L-lysine (PLL) microcapsules suppressed the growth of human glioma xenografts.¹³⁰ Alginate encapsulated human fetal kidney cells, transfected with a human endostatin-expressing vector, inhibited the growth of malignant brain tumors in rats.¹³¹ Recombinant fibroblasts expressing endostatin and encapsulated in PTFE capsules (Theracyte™) suppressed melanoma in mice.¹³² Melanomas were also suppressed when mouse myoblasts that were engineered to express angiostatin were encapsulated in alginate-PLL-alginate beads and grafted in tumor-bearing mice.¹⁵ Combinatorial cell therapy has improved outcomes by attacking multiple targets. For instance, cells expressing IL-2 and those expressing angiostatin were encapsulated and grafted in melanoma-tumor-bearing mice, resulting in suppressed tumor growth and enhanced survival in comparison to mice receiving monotherapy.^{133,134}

3. Cytokine Therapy—Tumors can also be suppressed using tumor-suppressing cytokines such as TNF- α , which results in tumor regression. However, TNF- α has high systemic toxicity with lower tolerance in humans than in murine species. Encapsulation can reduce systemic toxicity as explicated earlier; alginate-encapsulated J558 cells expressing TNF- α were effective in suppressing MCF-7 tumors in nude mice.¹³⁵

4. Clinical Trials of Encapsulated Cells in Cancer Therapy—A clinical trial to treat 14 patients with inoperable pancreatic cancer used cellulose sulfate (CapCell™) to encapsulate cells that were engineered to express the cytochrome P-450 2B1 enzyme.¹³⁶ This enzyme converts the ifosfamide prodrug to the cytotoxic metabolites phosphoramidate and acrolein. 300 capsules were angiographically delivered to each patient through an artery feeding the primary tumor. Ifosfamide was infused on 3 consecutive days starting on day 2 post implantation and the regimen was repeated on days 23–25. 4 patients showed >50% regression of tumor growth post treatment as compared with controls, and two others showed a 25–50% reduction in tumor volume. There was no further tumor growth in the remaining patients. The 1-year survival of 36% was triple that of controls and twice that of survival reported following gemcitabine treatment.

VI. CONCLUDING REMARKS -- CHOICES AND CHALLENGES FOR CLINICAL SUCCESS

The grafting of functioning cells is an attractive and achievable method to compensate for the loss of native cell mass or function. Cells sourced from other species, cells engineered

through genetic manipulation and stem cells can be used to restore physiology or to perform specialized and desirable tasks. However, the direct transplantation of these cells is undesirable in most cases because it can lead to either a coordinate immune response by the host or the uncontrolled proliferation and migration of the grafted cells. The immunoisulative encapsulation of cellular grafts can serve as a vehicle to deliver and sequester the cells at the target location without harm to graft and host. Macroencapsulation of cellular grafts affords ease of retrieval. By contrast, microencapsulated grafts are difficult to retrieve but they typically provide superior graft oxygenation. While microencapsulation in spherical alginate beads has been extensively applied in cell therapy, thin conformal polymer coatings on cells reduce transplant volume and provide superior response to rapid microenvironmental changes. However, these polymer coatings do not have precise control over their porosity, which is a prerequisite for the size-based exclusion of immune molecule penetration into the graft. Nanofabrication techniques such as nanolithography and nanoimprinting provide extreme precision over the porosity of the encapsulating membrane, but their utility in making a complete encapsulation device has not yet been realized because of limited control over the shape and the biofriendly sealing of such devices.

Encapsulated cells that secrete hormones and other small soluble factors can serve as a choice restorative for numerous acute and chronic diseases. For acute diseases, the encapsulating material may be biodegradable and grafted cells can be engineered with suicide genes, whereas chronic diseases may require cell graft regeneration or repeated graft infusions. Cell grafts may be engineered to perform specific functions under the control of tissue- or environment-specific promoters. This on-demand secretion or expression of molecules can be a potent tool against recurring diseases. For instance, encapsulated cells may reside in a tumor location and serve as a watchdog against recurrence, secreting anti-tumor molecules if recurrence is detected.

Encapsulated cell therapy cannot be used where cell-cell contact is necessary for appropriate graft function. Immunoisulative encapsulation is ineffective when the transport of large molecules is desirable and it does not preclude the transport of undesirable small molecules such as antigens shed by the graft. Grafts cannot be vascularized or innervated within immunoisulative capsules, and their homing and large-scale proliferation is precluded. With the aforementioned limitations, encapsulated cellular grafts present a safe option to treat cellular deficiencies on a wide scale and for a wide range of diseases.

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Figure 1.

Schematic representation of host immunorejection of cellular grafts, and its prevention through encapsulation. Left panel: Host immune system mediated rejection of transplanted cells through antigen-activated antibody response and through macrophage phagocytosis. Right panel: Immunoprotection of transplanted cells by (a) microencapsulation and (b) macroencapsulation through the physical isolation of the grafts and the prevention of direct contact with the macromolecules of the host immune system. Hydrogel permeability is directed by its cross-linking, and is characterized by a wide distribution of pore sizes, whereas nanoelectromechanical systems (NEMS) based microdevices have pore dimensions with nanometer precision.

Figure 2.
Scanning electron micrograph of a hydrogel (alginate) microbead.

Figure 3. Confocal fluorescence image of a conformally coated pancreatic islet. Reproduced with kind permission from Ref. 48. Copyright Wiley-VCH Verlag GmbH & Co. KGaA.

Figure 4.

Scanning electron micrograph of nanoporous membranes defined by electron beam lithography (a) and by nanoimprint lithography (b).

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Table 1**Candidate Diseases for Encapsulated Cell Therapy**

Disease	Encapsulated cells	Therapeutic Molecule	Ref
Hemophilia B	Human primary myoblasts	Human factor IX	[137]
Anemia	Myoblasts	Erythropoietin	[138]
Dwarfism	Fibroblasts	Porcine growth hormone cDNA (pGH cDNA)	[139]
CNS malignancies		Endostatin	[140]
Huntington's disease	BHK (Baby Hamster Kidney)	Ciliary neurotrophic factor	[141]
Hypothyroidism	Human parathyroid tissue	Parathyroid hormone	[142]
Liver disorder	Hepatocytes	-NA-	[143]
Chronic pain	Adrenal chromaffin cells	Catecholamines and endogenous opioids	[144]
Parkinson's Disease	Recombinant Baby hamster kidney (BHK) cells Recombinant PC12 cells	Glial cell line-derived neurotrophic factor (GDNF) L-DOPA and Dopamine	[145] [146]
Diabetes	Pancreatic islets	Insulin	[74–83]
Pancreatic cancer	Recombinant HEK 293 cells	Ifosfamide	[127–129]
Alzheimer's disease	Recombinant C2C12 myoblasts Human retinal pigment epithelial (RPE) cell line	Ciliary neurotrophic factor (CNTF) Nerve growth factor (NGF)	[147] [148]
Arthritis	Recombinant C2C12 murine myoblasts	Tissue inhibitors of metalloproteinases (TIMPs)	[149]
Retinal degeneration	Recombinant cells	Ciliary neurotrophic factor (CNTF)	[70, 71]