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Encapsulated cell therapy for neurodegenerative diseases: From promise to product☆

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ABSTRACT

Delivering therapeutic molecules, including trophic factor proteins, across the blood brain barrier to the brain parenchyma to treat chronic neurodegenerative diseases remains one of the great challenges in biology. To be effective, delivery needs to occur in a long-term and stable manner at sufficient quantities directly to the target region in a manner that is selective but yet covers enough of the target site to be efficacious. One promising approach uses cellular implants that produce and deliver therapeutic molecules directly to the brain region of interest. Implanted cells can be precisely positioned into the desired region and can be protected from host immunological attack by encapsulating them and by surrounding them within an immunoisolatory, semipermeable capsule. In this approach, cells are enclosed within a semiporous capsule with a perm selective membrane barrier that admits oxygen and required nutrients and releases bioactive cell secretions while restricting passage of larger cytotoxic agents from the host immune defense system. Recent advances in human cell line development have increased the levels of secreted therapeutic molecules from encapsulated cells, and membrane extrusion techniques have led to the first ever clinical demonstrations of long-term survival and function of encapsulated cells in the brain parenchyma. As such, cell encapsulation is capable of providing a targeted, continuous, de novo synthesized source of very high levels of therapeutic molecules that can be distributed over significant portions of the brain.

Contents

1. Introduction

Treating chronic, degenerative central nervous system (CNS) diseases is an urgent challenge. The most promising approaches propose to deliver neuroprotective/regenerative proteins directly to the site of cell loss to slow, halt, or even reverse the ongoing degeneration and associated

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neurological deficits. The development of effective neurotrophic molecules and proteins has been hindered in large part by the inability to deliver them across the blood brain barrier (BBB) directly to the target site in a stable, controlled, and continuous manner [1,2]. Several strategies are under development to optimize the diffusion and spread of trophic factors into the brain tissue. These include direct brain infusion, various gene therapy approaches, cell therapies, and biomaterial-based drug-delivery systems. As shown in Box 1, each approach has its own advantages and disadvantages. Encapsulated cell therapy overcomes many of the fundamental obstacles of other approaches by providing a targeted, continuous, de novo synthesized source of molecules that can be distributed over significant portions of the brain. In principle, this therapeutic approach combines the potency of de novo in situ synthesis of cell-derived proteins with the safety of an implantable and retrievable medical device. Cells are enclosed in a semipermeable capsule, which is implanted into the brain. The capsule is constructed from a hollow fiber membrane with unique isoreticulated pore structure that allows oxygen and nutrients to nourish the encapsulated cells while providing diffusive control of proteins and other molecules as they exit the capsule into the surrounding brain tissue. Immunological reactions against the encapsulated cells are obviated by the construction of the semipermeable thermoplastic membrane, eliminating the entry of the most damaging elements of the host immune system into the cell-containing lumen and thereby preventing rejection. This is accomplished by control of nominal pore size, distribution, tortuosity, and surface chemistry. By using human cells as the delivery platform, immunological reactions can be further limited to allogeneic involvement.

Owing to the physical integrity of the encapsulated cell capsule, in comparison to other hydrogel-based encapsulation techniques, an additional advantage of the technology is that the capsule can be configured so that it is easily removed and/or replaced if necessary or desired. For CNS applications, the use of conventional imaging and stereotactic procedures inherently provides a means of selectively targeting those areas of the brain where the secreted factor will be therapeutic. Because multiple implants can be used within the same target region it is possible to achieve far greater spread of protein throughout the targeted region than can be achieved with crude infusion of protein. This review discusses the pre-clinical and clinical evaluation of encapsulated cells for degenerative CNS diseases highlighting the therapeutic potential of genetically-modified, encapsulated cells for Alzheimer's disease (AD), Parkinson's disease (PD), and Huntington's disease (HD). We begin by detailing early studies that provided "proof of principle" for the concept of EC therapy and conclude with descriptions of recent efforts to optimize EC therapy into a viable therapeutic platform in functional and restorative neurosurgery.

2. Initial demonstrations of cell immunoisolation

Both micro-and macroencapsulation of cells have been investigated for CNS therapies but the majority of pre-clinical and virtually all clinical work to date has focused on macroencapsulation (Tables 1, 2). In macroencapsulation, pre-fabricated devices with internal cell-supportive scaffolds are loaded with cells suspended in culture medium or an isotonic buffer, and sealed to form a capsule. Initial studies supported the promise of immunoisolation in vitro by measuring the retention of polydisperse dextran solutions across a semi-permeable capsular membrane or the in vitro protection of encapsulated cells against the cytotoxic killing of antibody (IgG)-mediated complement lysis [93,94]. While useful, in vitro analyses do not provide the essential demonstration that cell immunoisolation maintains cell viability and function in vivo. The importance of polymer capsule integrity was confirmed in early studies using xenografted tissue where unencapsulated cells, or cells encapsulated within intentionally damaged membranes, were implanted into guinea pig or monkey brains [54,95,96]. While unencapsulated cells or cells loaded into damaged devices were rapidly rejected, cells in intact capsules remained viable. The surrounding tissue was also devoid of characteristic immunological reactions such as lymphocytic infiltration and astrocytic reactivity that signifies the rejection of unencapsulated cells or cells within damaged devices. These initial studies therefore also highlighted the biocompatibility of encapsulated cells, which is an obvious issue in maintaining the survival of the cells. The lack of a significant host tissue reaction to the implant is crucial for both initial and long-term viability of the encapsulated cells. While capillary invasion helps provide nutrients and oxygen in proximity to the encapsulated cells the process of

Box 1
Approaches for drug delivery to the brain.

Delivery of molecules to the brain is limited following systemic administration largely because the blood–brain barrier modulates exchange between the vasculature and brain parenchyma [1,2]. This modulation makes it difficult, if not impossible, to achieve therapeutic concentrations of potentially effective molecules such as trophic factor proteins within the desired site of the brain. Several approaches are under investigation for direct, local delivery of these compounds to the desired brain target. These include:

Direct brain infusion. A catheter implanted into the brain is attached to a pump to control the rate and timing of infusion. The surgery is invasive, the pumps are prone to leakage, protein stability can be poor, immunological responses can block protein function, and distribution from a point source is inadequate [3–6].

Gene therapy approaches. A viral vector containing the gene that expresses a factor is injected into the brain causing the local neurons to produce the factor. This approach can achieve localized factor production but there isn't any way to regulate expression or stop the treatment once the virus is injected raising safety and regulatory concerns [7–9].

Cell-based delivery. Cells (e.g stem cells) engineered to produce trophic factors are injected into the brain where they can migrate to increase diffusion of the desired factor into the brain tissue [10]. This approach may permit anatomical integration between the host and transplanted cells and good cell viability and neurochemical diffusion may be achieved. Migration is uncontrolled, the cells cannot be retrieved, and the patients' bodies may reject the stem cells.

Biomaterial-based drug delivery. Molecules can be incorporated into injectable or implantable biomaterials to provide sustained local targeted delivery to the brain, allow cell or tissue transplants to be delivered to the brain and help promote regeneration of damaged neuronal pathways [11]. These approaches do not currently provide long-term delivery suitable for chronic CNS diseases and require significant experimental advancements to become clinically viable.

Cell encapsulation. There are generally two categories for cell immunoisolation by encapsulation, micro- and macro-, each with some benefits and limitations [12–16]. Microencapsulation permits use of allo- and xeno-grafts without immunosuppression and the thin wall and spherical shape are optimal for cell viability and neurochemical diffusion. These same capsules are, however, mechanically and chemically fragile and cannot be retrieved once implanted within the brain parenchyma. Macrocapsules also permit the use of allo- and xeno-grafts without immunosuppression, provide good cell viability and neurochemical diffusion, have good mechanical stability, and can be retrieved if needed or desired. Multiple implant sites may be required for optimal benefits.

Other. Other approaches to brain drug delivery such as carrier-, or receptor-mediated transcytosis [17,18] and osmotic opening [19,20] have been explored but have met with limited success. These approaches also are limited to drugs without systemic toxicity.

Table 1
Examples of cell encapsulation for CNS and CNS-related diseases.

Disease/model	Cells/experimental paradigm	Results and references
Age-related/neurodegenerative diseases		
Age-related motor decline	Catecholamine and GDNF cells in rats	Improvement in motor function [21–23]
Amyotropic lateral sclerosis	CNTF producing cells in mice	Protection of motor neurons [24]
Alzheimer's disease	NGF cells in rat and primates	Protection of cholinergic neurons, improved memory [25–29]
	CNTF cells in transgenic mice	Improved cognition, synaptic stabilization [30]
	VEGF cells in transgenic mice	Improved cognition, decreased Abeta and tao burden [31]
Epilepsy	BDNF/GDNF cells in rats	Decreased seizures, neuroprotection [32,33]
Huntington's disease	NGF and CNTF cells in rat and primates	Neuroprotection, improved behavior [34–40]
	Choroid plexus epithelial cells	Neuroprotection, improved behavior [41–46]
Parkinson's disease	Catecholamine and trophic factor cells in rat and monkey	Improved behavior, neuroprotection [47–63]
	Choroid plexus epithelial cells	Neuroprotection, improved behavior [64]
Retinitis pigmentosa	CNTF cells into the vitreous	Rod preservation [65]
Spinal cord damage	BDNF cells in rats	Axonal growth, improved motor function [66]
Glioblastoma	Endostatin, antibody-secreting, single chain-TRAIL and cells in mice and rats	Reduced tumor growth, enhanced survival [66–78]
Acute and chronic pain	Chromaffin cells in rats	Reduced pain [79–82]
Clinical trials		
Amyotropic lateral sclerosis	CNTF cells intrathecally	Sustained delivery, no toxicity [83,84]
Alzheimer's disease	NGF cells	Safe, sustained NGF delivery [85,86]
Chronic pain	Chromaffin cells in subarachnoid space	Safe but no pain reduction in phase II trials [87–89]
Huntington's disease	CNTF cells	Safe, limited cell viability [90]
Retinitis pigmentosa/geographic atrophy	CNTF cells in eye	Safe, long-term CNTF secretion, no efficacy [91,92]

angiogenesis for neovascularization evolves over several days. Therefore, the encapsulated cells must endure an initial period of nutrient and oxygen deprivation obtaining these essential factors only by diffusion. The long-term survival of encapsulated cells could also be negatively impacted by any local reaction that restricts nutrient flow from the brain tissue to the encapsulated cells. Moreover, since delivery of the desired cellular products from the encapsulated cells is by diffusion, any reaction around the capsule could limit the diffusion from the capsule. Together, these early studies demonstrated the concept of immunoisolation and biocompatibility within the host nervous system suggesting that the bidirectional transport of low molecular weight solutes across the membrane can be maintained in vivo. Interestingly, the CNS may in some ways be a more forgiving implant site for certain encapsulation systems. For instance, microencapsules have been investigated for delivery of molecules such as insulin within the periphery for many years. By their very nature, these capsules need to be implanted, in large quantities, into environments such as the abdomen where cell survival is greatly impacted by mass transport, diffusion, cellular overgrowth, and direct/indirect immune recognition. While challenges still exist using these approaches in the brain, the smaller numbers of capsules potentially needed along with somewhat better nutrient and oxygen availability in tissue and CSF implant sites could potentially overcome some frequently encountered issues that frequently occur with peripheral systemic implantation of microcapsules into larger compartments.

3. Long-term product secretion and delivery

By their very nature, chronic neurodegenerative diseases require long-term treatment which makes the extended survival of the encapsulated cells and continued release of the therapeutic molecule essential. Cells placed within encapsulation devices generally fall into one of three categories. The first category is represented by primary post-mitotic cells such as islets of Langerhans for diabetes, adrenal chromaffin cells for chronic pain or hepatocytes for liver devices. Secondly, immortalized (or dividing) cells such as PC12 cells have been utilized to deliver dopamine for PD. The third category is typically cell lines that have been genetically engineered to secrete a bioactive substance such as fibroblasts or ARPE-19 cells to secrete factors such as nerve growth factor (NGF) for a potential therapy in AD. Dividing cells have advantages over post-mitotic tissue as they can be expanded, banked and thus more easily tested for sterility and contaminants. However, because some cells (fibroblasts or PC-12 cells) will continue to divide once encapsulated and implanted, their use can be constrained by the potential for overgrowth within the capsule environment, resulting in an accumulation of necrotic tissue and potentially diminished membrane permeability characteristics, further reducing cell viability and neurochemical output. Cell lines such as ARPE-19 cells have been used both pre-clinically and clinically and have several inherently desirable characteristics including being of human origin with reduced potential for zoonosis and immunological rejection and contact inhibition that limits the potential for overgrowth once encapsulated and implanted.

Although surprisingly few studies have examined implant viability for more than a few months there is compelling evidence about the potential for long-term survival and release of molecules from the cells. Encapsulated PC12 cells have survived in vivo for up to 6 months, while maintaining a typical morphology and production of catecholamines [50,54,55,95]. Other cell types, including encapsulated bovine adrenal chromaffin cells, also survive for 1.5 years with continued production of catecholamines and met-enkephalin [80,96,97]. Polymer-encapsulated, genetically-modified cells also survived and continued to secrete trophic factors such as nerve growth factor (NGF) for 1 year in rats [98,99]. The cells remained viable and the NGF secreted from

Table 2
Advantages and disadvantages of unencapsulated, microencapsulated, and macroencapsulated cell implants.

	Advantages	Disadvantages
Unencapsulated (e.g. stem) cells or tissue	Allows anatomical integration with host, suitable cell viability and diffusion, in some cases can engineered.	May require immunosuppression tissue availability may be limited, cannot be retrieved, and may encounter societal and ethical issues.
Microencapsulated cells	Able to use allo- and xeno-grafts without immunosuppression, thin wall and shape are good for cell viability and diffusion.	Mechanically and chemically fragile, multiple implant sites likely required for CNS, and difficult to retrieve.
Macroencapsulated cells	Able to use allo- and xeno-grafts without immunosuppression, good mechanical stability, cell viability and diffusion adequate, and devices can be retrieved.	Dimensions can limit diffusion and cell viability, multiple implants are likely required for CNS, and tissue displacement can occur during implantation.

the encapsulated cells remained comparable to pre-implant levels. One limitation of the published studies to date is that they only compare pre-implant secretion to post-explant secretion. While this is important, this limited snapshot does not capture any potential fluctuations in secretion and it is largely unknown what the short, intermittent, or long-term pharmacokinetic profiles look like following implantation in any CNS site. This issue is also tightly related to the ability to control the dosing of factors released from encapsulated cells. While many interesting possibilities for dose control exist including modifying the size of device, controlling the numbers of cells within the device, or the method of cell transfection these approaches are all difficult to evaluate without adequate knowledge of the desired result.

These early studies provide pivotal support for the notion that encapsulated cells can survive and function for long periods of time when implanted into the brain. They also, however, illustrate that secretion and diffusion of cellular products can be limited. Tresco et al. showed that encapsulated PC12 cells can secrete dopamine into the denervated striatum of rats at a level high enough to induce behavioral recovery [50]. However, microdialysis in these same animals failed to demonstrate that dopamine diffused more than 200 μm from the capsules. Immunocytochemical analysis showed that the diffusion of NGF from encapsulated cells was approximately 1 mm in the striatum of rats [26]. Further, cerebrospinal fluid levels of NGF were below detectable levels in nonhuman primates [27] that received intraventricular grafts of NGF-producing cells suggesting that at best, secretion and diffusion of molecules from encapsulated cells can be limited, indicating interesting and significant proof of concept while demonstrating the need for further research and development to enable clinical translation.

4. Therapy using encapsulated cells: Parkinson's disease

Parkinson's disease (PD) is a progressive, idiopathic degenerative disorder of the CNS characterized by severe, debilitating motor impairments resulting from the dysfunction and loss of dopamine-secreting neurons in the substantia nigra. Pharmacologic treatments such as levodopa and dopaminergic agonists provide symptomatic relief of the motor symptoms early in PD by compensating for the loss of dopamine transmitter capacity. Initial proof of concept data for the use of encapsulated cells in PD came from studies in both rodents and primates demonstrating that encapsulated PC12 cells implanted into the striatum deliver L-dopa and dopamine in sufficient quantities to induce behavioral improvements [49–53]. While L-dopa is remarkably effective for many years in PD patients, its effectiveness is only temporary and continued loss of dopaminergic neurons eventually leads to a point when these drugs become ineffective. An ideal treatment for PD would be one that restores the function of dopaminergic neurons while also protecting them from further damage and loss. Accordingly, trophic protein factors are under intensive investigation as such a treatment due to their inherent roles in promoting neuronal survival, growth and function.

Several studies have demonstrated the benefits of encapsulated neurotrophic factor-secreting cells in rodent models of PD. GDNF and its family member neurturin are particularly interesting molecules in the context of treating PD. GDNF is a small protein that potently promotes the survival and differentiation of dopaminergic neurons both in vitro and in animal models of PD. Like most neurotrophic factors, GDNF cannot be effectively delivered to the brain via systemic administration but when delivered directly to the brain it does prevent the loss of nigral neurons and abnormal motor function that occurs following 6-hydroxydopmine lesions in rats [100,101] and MPTP-lesioned monkeys and enhances dopaminergic function in aged monkeys [102–104]. Together, these data make a strong case for testing the ability of GDNF to both protect dopaminergic neurons from ongoing degeneration as well as potentially induce the regeneration of dopaminergic fibers in the denervated brain. GDNF has been tested in PD patients but with inconsistent results. The lack of reliable benefits in patients is likely a result of poor penetration of GDNF to the nigrostriatal system when delivered non-specifically from the intraventricular space [105] or limited distribution within the brain parenchyma when delivered via a single point source from pumps [106–109]. Together, these data indicate that a prominant, limiting factor in the development of GDNF is a delivery approach that provides long-term, delivery of sufficient quantities of GDNF to the nigrostriatal system in a manner that is selective but yet covers enough of the nigrostriatal system to be effective. Cell encapsulation may be one means of achieving these goals. Encapsulated cells releasing approximately 5 ng of GDNF/day were implanted immediately rostral to the substantia nigra [61]. The medial forebrain bundle was transected one week later and the ability of encapsulated GDNF-producing cells to minimize the behavioral effects of the lesion and prevent the degeneration of dopaminergic neurons was determined. GDNF significantly reduced amphetamine-induced rotations in lesioned animals and attenuated the loss of neurons in the substantia nigra but had no effect on dopamine within the denervated striatum. Using the same model, a neurturin-producing cell line, a homologue of GDNF, was investigated for its neurotrophic activity [62]. Neurturin-treated animals had significantly more TH-positive neurons in the substantia nigra (51% compared to 16% in controls) but failed to show any behavioral improvement as measured by rotational behavior. Together, these data suggest that encapsulated cells may have a role in neurotrophic therapy for PD. However, the low levels of GDNF and Neurturin delivered in these studies were likely too low to exert any beneficial effects in PD patients due to limited diffusion and exposure. Investigation of new generation devices with higher GDNF secretion and tissue levels is therefore warranted.

Other pre-clinical PD studies have used alginate-microencapsulated cells to deliver GDNF [110–113] and the VEGF [114–116]. In one of these studies, encapsulated GDNF-secreting fibroblasts were implanted into the striatum of 6-hydroxydopamine (6-OHDA) injured rats. Long-term expression of GDNF, up to 6 months, was detected along with behavioral improvement and tissue biocompatibility of the capsules [111]. The benefits of this approach were more pronounced when the morphogens were delivered at the time of the injury rather than weeks or months later, suggesting a neuroprotective rather than restorative effect. In another study, porcine choroid plexus cells were encapsulated in alginate-poly-L-ornithine-alginate capsules and implanted them into the striatum of 6-OHDA lesioned rats. Results showed that the presumed neurorestorative proteins secreted by the encapsulated cells improved motor behavior and nigrostriatal dopaminergic activity [64,117].

A frequently overlooked potential use of encapsulated cell therapy is as a means of delivering trophic factors to the brain to support the survival of co-grafted cells. In a series of studies, baby hamster kidney (BHK) cells were genetically-modified to secrete NGF. Following encapsulation, these cells were implanted into the lateral ventricle or striatum approximately 1.5 mm away from co-grafted unencapsulated rat chromaffin cells in hemiparkinsonian rats [118,119]. Although the animals receiving adrenal medulla alone or adrenal medulla with intraventricular NGF-secreting cell grafting did not show recovery of apomorphine-induced rotational behavior, the animals receiving adrenal medulla with intrastriatal NGF-secreting cell implants showed a significant recovery of rotational behavior. Histological analysis revealed that intraventricular NGF increased the number of surviving chromaffin cells five to six times above that seen in animals receiving adrenal medulla alone. Even more impressively, intrastriatal NGF-secreting cells increased the number of surviving chromaffin cells by more than 20 times higher than in animals receiving adrenal medullary cells alone. Additional studies determined that the beneficial effects of NGF-producing cells were evident for as long as 12 months post-grafting [98]. Similar effects were reported by Ahn et al. [120], who noted enhanced fiber outgrowth from grafted human embryonic dopaminergic neurons when co-grafted with encapsulated GDNF-secreting C2C12 cells. These results support the potential use of encapsulated trophic factor-secreting cells for augmenting the survival of co-grafted cells and raise interesting possibilities regarding the use of co-grafted encapsulated cells to control

the survival, differentiation, and migration of cells; including stem cells, in vivo. Once again, though they also highlight the importance of intraparenchymal delivery and the potential consequences of poor distribution secondary to low dose factor secretion.

5. Therapy using encapsulated cells: Alzheimer's disease

Alzheimer's disease (AD) is the most prevalent form of adult onset dementia. The most prominent feature of AD is a progressive deterioration of cognitive and mnemonic ability, which is at least partially related to the degeneration of basal forebrain cholinergic neurons. At present, treatments do not slow or prevent cholinergic neuron loss or the associated memory deficits. NGF has potent target-derived trophic and tropic effects upon cholinergic basal forebrain neurons and is being explored as a potential treatment. Although no model faithfully recapitulates AD, models of cholinergic cell loss are used to determine if therapeutic molecules (NGF or others) prevent the death of damaged cholinergic neurons following trauma. Endogenously, NGF is retrogradely transported from cortical and hippocampal regions to the cholinergic cell bodies in the basal forebrain. In AD patients this transport is significantly reduced and while the mechanisms behind this reduction are not fully understood, these changes may be pivotal for events in the decline in cholinergic function in patients. Given these considerations, delivery of NGF directly to the basal forebrain is being actively investigated as a means of preventing the otherwise relentless loss of cholinergic neurons in AD patients. Initial studies demonstrated that implants of encapsulated NGF-secreting BHK cells prevented cholinergic neuron loss following aspiration of the fimbria/fornix. Control-implanted animals had an extensive loss (88%) of ChAT-positive cholinergic neurons ipsilateral to the lesion that was prevented by NGF cell implants (14% loss) [28]. Aged rodents can also be used to examine the benefits of NGF on cognitive function. Lindner et al. trained 3, 18 and 24-month old rats on a spatial learning task in a Morris water maze. Cognitive function, as measured in this task declined with age [25]. Following training, animals received bilateral intraventricular implants of encapsulated NGF or control cells. The 18 and 24-month old animals receiving NGF cells significantly improved on the task without alterations in mortality, body weights, somatosensory thresholds, potential hyperalgesia, or activity levels, suggesting that the levels of NGF produced were neither toxic nor harmful to the aged rats. Anatomically, the NGF released from the encapsulated cells increased the size of the atrophied basal forebrain and striatal cholinergic neurons to the size of the neurons in the young healthy rats.

Results similar to those obtained in rodents were obtained in nonhuman primates. Cynomolgus primates received transections of the fornix followed by placement of encapsulated NGF-secreting or control cells into the adjacent lateral ventricle [27]. In controls, a significant reduction in the number of cholinergic neurons occurred in the medial septum and vertical limb of the diagonal band of Broca that was prevented by NGF. The cholinergic neurons within the medial septum of NGF-treated animals were larger, more intensely labeled, and elaborated more extensive proximal dendrites than in controls. Encapsulated NGF implants induced a robust sprouting of cholinergic fibers proximal to the implant site. Dense collections of cholinergic fibers were seen throughout the dorso-ventral extent of the ipsilateral lateral septum. These fibers ramified against the ependymal lining of the lateral ventricle adjacent to the transplant site and were particularly prominent within the dorsolateral quadrant of the septum corresponding to the normal course of the fornix. The cell sparing and sprouting were replicated in a group of aged nonhuman primates [29].

Several new studies in experimental AD models have tested the use of cell microencapsulation technology. When encapsulated cells secreting CNTF were implanted intra-cerebroventricularly into mice expressing the mutant amyloid precursor protein a significant improvement in cognitive function occurred. In other studies, encapsulated mesenchymal stem cells (MSC) expressing glucagon-like peptide-1 (GLP-1) were evaluated in an AD double transgenic murine model for their ability to GLP-1 decrease amyloid deposition and A-beta-induced toxicity [121–124]. Results suggested a reduction in A-beta-induced toxicity in vitro along with anti-inflammatory and neuroprotective properties [124]. Other studies have shown that (1) implants of microencapsulated VEGF-secreting cells improve cognition, reduce brain Ab burden, apoptotic cell death and hyperphosphorylated-tau expression [125] in transgenic (APP/PS1) mice, (2) that this treatment enhance cellular proliferation in the dentate gyrus and reduce the expression and activity of acetylcholinesterase in the same mouse model [126], and (3) that microencapsulated CNTF-secreting cells improve cognition and stabilize synaptic proteins in Abeta oligomer-infused and Tg2576 mice [30].

6. Therapy using encapsulated cells: Huntington's disease

Huntington's disease (HD) is an inherited, progressive neurological disorder characterized by severe degeneration of basal ganglia neurons, particularly the intrinsic neurons of the striatum. At present there is no treatment that effectively addresses the behavioral symptoms or slows the inexorable neural degeneration in HD. The use of trophic factors in a neural protection strategy may be particularly relevant for the treatment of HD where genetic screening can identify individuals at risk providing a unique opportunity to design treatment strategies to intervene prior to the onset of striatal degeneration. Of the neurotrophic factor studies to date, CNTF appears to be one of the most promising candidates in HD. Ciliary neurotrophic factor is a protein encoded by the CNTF and is a polypeptide hormone with potent effects on several cell populations including neurons, astrocytes, oligodendrocytes, and retinal cells. CNTF has been evaluated in clinical for ALS as well as ongoing trials for retinitis pigmentosa. As is the case with other proteins, CNTF crosses the BBB poorly and needs to be delivered directly to the brain to have the opportunity to exert any potential benefits. In the context of HD, direct local administration of CNTF potently protects the striatal neurons that die in HD and has led to the speculation that if delivered appropriately it might similarly slow or halt the neuronal degeneration that occurs in patients. Using the quinolinic acid model of HD, rats received implants of NGF- or CNTF-producing cells followed 1 week later by injections of QA into the ipsilateral striatum [34–36]. An analysis of Nissl-stained sections demonstrated that the size of the lesion was significantly reduced by NGF and CNTF. Moreover, CNTF cells spared important populations of striatal cells, including cholinergic, diaphorase-positive and GABAergic neurons. Importantly, behavioral studies illustrated improved performance on learning and memory tasks, indicating the anatomical protection afforded by trophic factors in this model is paralleled by a robust and relevant behavioral protection. These experiments led to similar studies in nonhuman primates. Polymer capsules containing CNTF-producing cells were grafted into the striatum of monkeys followed 1 week later by QA injections proximal to the capsule implants [37]. As a result of CNTF treatment, the volume of striatal damage was decreased and both GABAergic and cholinergic neurons were spared. Although all animals had significant lesions, there was a 3-fold and 7-fold increase in GABAergic neurons in the caudate and putamen, respectively in CNTF grafted animals relative to controls. Similarly, there was a 2.5-fold and 4-fold increase in cholinergic neurons in the caudate and putamen, respectively in CNTF-grafted animals. Subsequent analyses revealed that not only was the intrinsic striatal cytoarchitecture preserved by CNTF, but the striatal cells also maintained their projections and their cortical afferents. Encapsulated CNTF producing cells were also therapeutic in 3NP-treated monkeys [38]. Following 10 weeks of 3NP treatment, monkeys displayed pronounced chorea and severe deficits in frontal lobe cognitive performance as assessed by the object retrieval detour test. Following implantation of encapsulated CNTF-producing cells, a progressive and significant recovery of motor and cognitive recovery occurred. Histological analysis demonstrated that CNTF was neuroprotective and spared NeuN and

calbindin-positive cells in the caudate and putamen. While the sparing of striatal neurons and maintenance of intrinsic circuitry in these studies are impressive, the magnitude of the effect is less than that seen in rodents. In primates, robust protection is limited to the area of the capsules and the total area of the lesion remains extensive; likely because the diffusion of CNTF from the capsule is not sufficient to protect more distant striatal regions undergoing degeneration. This concept is supported by a recent experiment examining the effects of intraventricular grafts of encapsulated CNTF grafts in the nonhuman primate model of HD. In contrast to capsule placement directly within the brain parenchyma, intraventricular placements failed to engender neuroprotection for any striatal cell type [39,40].

Clinical trials were initiated to determine the safety and tolerability of CNTF-producing cells implanted into the lateral ventricle of HD patients. Six patients had a single capsule implanted into the right lateral ventricle and the capsule was retrieved and replaced every 6 months over a 2 year period [90]. No signs of CNTF-induced toxicity or efficacy were observed and while all retrieved capsules were intact, CNTF secretion was low and the explanted devices contained low numbers of surviving cells. If human trials are to yield clinically relevant positive effects, the means of CNTF delivery needs to be improved. Whether this entails changing the site of implantation from the ventricle to the parenchyma, grafting more capsules, enhancing the CNTF delivery from the cells by changing the vector system or cell type employed, or changing the characteristics of the polymer membrane remains to be determined.

7. Shifting from promise to clinical therapy

Cell encapsulation has been and remains an intuitively appealing means of achieving one of the holy grails of biology: enabling the effective delivery of efficacious compounds to the diseased brain to treat neurodegenerative diseases. From its conception, cell encapsulation technology seemed capable of allowing site specific delivery of potent molecules for prolonged periods of time. At the same time it was clear that these results were inconsistent enough to limit clinical development of this technology despite significant efforts in both academic laboratories and biotechnology companies. There were, however, several research and development pathways that emerged that have yielded significant improvements in encapsulation technology and have produced what now appears to be an achievable, clinically viable platform technology for restorative and functional neurosurgery and the treatment of degenerative brain diseases (Box 2). These improvements include the following.

7.1. Human cell line development

The majority of cell encapsulation studies to date have used proliferating or terminally differentiated animal cells. Animal-derived fibroblasts, for example, are readily available and can be genetically-modified to secrete trophic proteins they are also difficult to control once encapsulated and can even overgrow the capsular environment, form necrotic cores, and survive unpredictably in the long-term. While long-term survival in CSF-filled implant sites has been variably observed [25,27–29,99], viability of these cells has been more limited when implanted into the brain tissue and most studies have been short-term demonstrations of functional or anatomical benefits. Recently, a human cell line (ARPE-19) has been used as a platform cell line for both pre-clinical and clinical evaluation. Because these cells are allogeneic, the overall risk of both zoonosis and immune reactions are reduced when implanted into human patients. Moreover, these cells are considerably hardy since they can survive under stringent conditions such as implantation into the eye, or even in vitro in extreme conditions of nutrient deprivation. Indeed, encapsulated ARPE-19 cells have survived for at least 2 years in the human vitreous humor [92] and at least 1 year in the brain parenchyma of Alzheimer's patients [85,86]. The cells can, in principle be modified to produce any trophic factor and have successfully been used to deliver CNTF [65,92] and NGF [85,86,127–129] both pre-clinically and clinically. These cells have also been shown to be non-tumorigenic and from a practical point of view are long-lived with progenies that can be cloned and banked under rigorous GMP procedures.

Box 2
Inspiration, evolution, and transition of cell encapsulation into therapy.

Cell encapsulation is an intuitively appealing means of treating CNS diseases by enabling sustained, local, and efficacious delivery of trophic factors and other molecules to the brain parenchyma. Despite establishing many of the essential pre-requisites of this technology in animal models during the 1990's, the field had difficulty living up to expectations, and clinical evaluation yielded promising but insufficient results.

Where we were. It was evident early on that encapsulated cells could be configured into implantable capsules that were biocompatible, were capable of maintaining cell viability for extended periods of time, and were functional in animal models of CNS diseases. While demonstrating these principles and conducting a few small Phase I clinical trials it also became evident that these configurations produced results that were inconsistent enough to limit clinical investigation and that additional technical development was needed. The cells that were used (e.g. modified fibroblasts) were nonhuman, animal-derived cells that represented uncontrolled dividing tissue that frequently overgrew the capsule environment, resulting in an accumulation of necrotic tissue that diminished the membrane's permeability characteristics, further reducing cell viability and secretion. The cells and the techniques used for genetic modification resulted in factor secretion that was very low, provided limited diffusion within the brain tissue, and made the prospects for adequate distribution in the human brain difficult to achieve. The membranes used were generally biocompatible but also permitted tissue ingrowth into the membrane walls that had the potential to further limit bi-directional diffusion while also raising possible concerns during device retrieval. Despite these setbacks, relatively clear pathways emerged for transitioning this technology into a viable clinical product.

Where we are. The old adage "good things take time" might very well apply to cell encapsulation. Encapsulated cell technology has moved beyond animal cells and now utilizes human cells that have demonstrated excellent long-term viability and function in animals and humans. Membranes and cell scaffolds have also been developed under rigorous, well-controlled manufacturing processes further augmenting the continued survival and function or encapsulated human cells. Moreover, advances in molecular biology have increased the secretion of therapeutic molecules from encapsulated cells by several log orders. This is a particularly important point since CNS degenerative diseases are not treatable by delivering drugs systemically or from the ventricular space given that diffusion of compounds in the brain tissue is severally limited when governed only by passive diffusion. Finally, the development and continued refinement of clinical implantation systems compatible with conventional stereotactic techniques ease the transition from pre-clinical evaluation into widespread clinical implementation.

7.2. Device manufacturing and scaffold development

From a mechanical perspective, the three most important aspects of a cell encapsulation device are the membrane, the adhesive that forms the seals, and the extracellular matrix within the membrane. While all 3 elements are in physical contact with the cells encapsulated within, both the membrane and the adhesive are directly exposed to the implant environment. The membrane provides an element of torsional

support and allows for appropriate diffusion of molecules from the cells across the membrane and into the adjacent brain tissue. Several membrane materials have been contemplated including poly(acrylonitrile), poly(sulfone), poly(ether sulfone; PES), poly(amides), poly(propylene), and poly(ethylene). Most work within the CNS has revolved around the use of poly(acrylonitrile-co-vinyl chloride; PAN-PVC). PAN-PVC membranes do have excellent in vivo stability and can be prepared with appropriate mechanical strength and transport properties. They can also support cell survival when implanted into CSF-filled locations but tend to be modestly compatible within brain tissue. In several studies, tissue ingrowth was evident upon histological evaluation of explanted devices increasing the chances of device breakage upon removal while decreasing the opportunity for adequate bi-directional diffusion across the membrane in vivo. These issues appear to have been adequately resolved in recent studies using PES membranes. NGF-secreting cells loaded within PES membranes were implanted into the brains of minipigs and were successfully explanted 12 months later [128]. All of the devices were removed intact with no tissue adherence and viable cells were identified with continued secretion of NGF. These studies also illustrate the importance of the cell scaffolding within the capsule. It has long been known that the survival and differentiation of encapsulated cells can be influenced by matrix interactions. In vivo, extracellular matrices (ECMs) control cell function through the regulation of morphology, proliferation, differentiation, migration and metastasis. In the context of cell encapsulation, ECMs were originally employed to prevent aggregation of cells (immobilization) and resultant central necrosis, but were subsequently found to be beneficial scaffolds for cell viability and function of anchorage-dependent cell lines. For example, adrenal chromaffin cells have been immobilized in alginate to prevent aggregation that, in turn, reduces central necrotic cores from forming [48]. In contrast, BHK cells, a fibroblastic cell line, prefer collagen, while PC12 cells exhibit a preference for distribution within precipitated chitosan, which provides a scaffolding structure on which the cells anchor [51]. With ARPE-19 cells the replacement of a polyvinylalcohol foam scaffolding with a polyethylene terephthalate (PET) yard significantly improved cell viability and function while also making the manufacture of the devices more reproducible (Fig. 1). A recent clinical trial involving encapsulated ARPE-19 cells to deliver NGF to the brains of Alzheimer's patients confirms the promise of these recent advances [85]. One centimeter-long capsules were attached to an inert polymer tether and placed bilaterally into the basal nucleus of Meynert (3 patients) or both the basal nucleus of Meynert and the vertical limb of the diagonal band of Broca. Post-operative CT scans confirmed appropriate placement of the capsules and MRI images at 3 and 12 months showed no evidence of inflammation or device displacement. At 12 months, implants were successfully retrieved intact, and low but persistent NGF secretion was detected in half of the patients. The importance of selecting a sealant with minimal unreacted residuals and a biocompatible surface chemistry was highlighted in early device configurations that suffered from cellular efflux, a biomaterial foreign body reaction, or the focal attachment of host fibroblast to the seals themselves. From a device manufacturing perspective, it is the ability to control the flow of the sealant by selecting an appropriate viscosity that is the most important aspect, while it must maintain a strong bond in vivo by penetrating within the matrix of the cell scaffold material as well as the membrane. Photopolymerizable urethane acrylate oligomers have been used for this purpose, which provide the requisite surface tension, viscosity, and have demonstrated unmatched biocompatibility in numerous studies [91,92,128,129].

7.3. Enhanced secretion and distribution of trophic factors

Delivery of trophic factors via encapsulated cells has enjoyed relatively robust and consistent success in animal models ranging from rodents to nonhuman primates. These studies, however, have made it clear that intraparenchymal delivery in the human brain would require

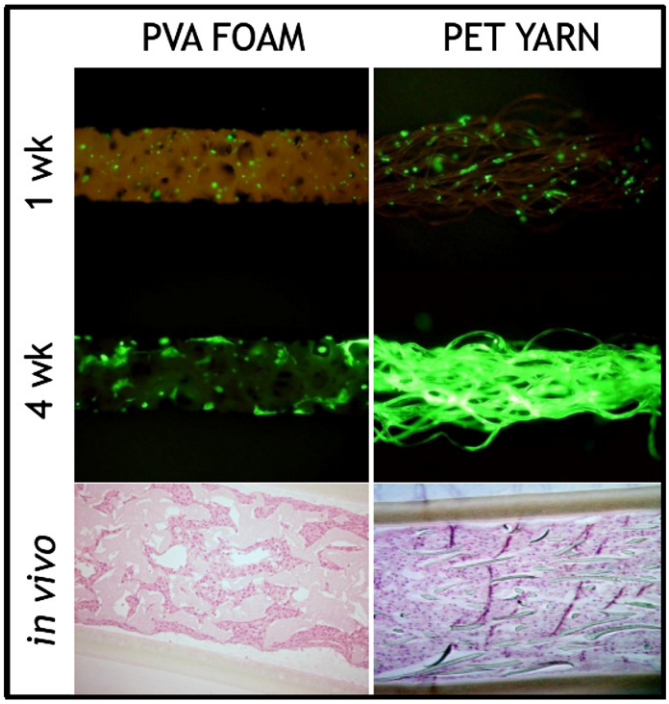

Fig. 1. Illustration of how the optimization of the membrane and scaffold components of cell encapsulation devices can profoundly augment the in vitro and in vivo performance of encapsulated cells. ARPE-19 cells expressing green fluorescent protein show excellent viability when encapsulated into PES membranes containing PET yarn as an internal scaffold material. As early as 1 week in vitro the cells are homogeneously distributed throughput the device and can be seen elongating along the yarn material. At 4 weeks, cells can be seen in both within the foam scaffolding and the yarn scaffolding but the viability and cell proliferation are clearly superior when the cells are encapsulated with the yarn scaffold. H&E histology from devices explanted from rat brains confirms that this configuration maintains excellent cell viability in vivo.

significantly higher factor secretion to enable adequate distribution throughput the target region. To accomplish this goal, clonal human ARPE-19 cell lines producing NGF were generated using the Sleeping Beauty transposon system [127–129]. This process resulted in stable gene transfer with long-term expression and bioactivity both in vitro and in vivo. NGF levels were approximately 10 times greater than that achieved previously using other constructs. Notably, when implanted into minipig brain the resulting diffusion of NGF in the target region (basal forebrain) was substantial [129], yielding diffusion distances from the implant sites up to 2.5 mm as judged immunohistochemically. Previous studies have demonstrated that immunohistochemistry likely underestimates the distance that biologically active trophic factors such as NGF diffuse in the brain [26] making it probable that the measured 2.5 mm of diffusion is a low estimate in these studies. Cell lines have also recently been generated that secrete GDNF levels approaching microgram quantities per day ([130]; in press; Fig. 2) making it conceivable for the first time that GDNF can be delivered in a sustained manner that covers the putamen of Parkinson's patients. Even with significantly enhanced secretion of factors such as GDNF, multiple implant sites will still likely be required to deliver enough factor to cover the target region in a human brain. Multiple surgical implants should not be either a surgical or safety concern though as clinical trials have already incorporated the use of 4 implants into the brain of AD patients. Additional studies would of course be needed to be certain that increasing the number of implants did not raise any safety concerns.

8. Conclusion

The use of polymer encapsulated cells has always been an intuitively appealing means of overcoming the blood brain barrier to deliver

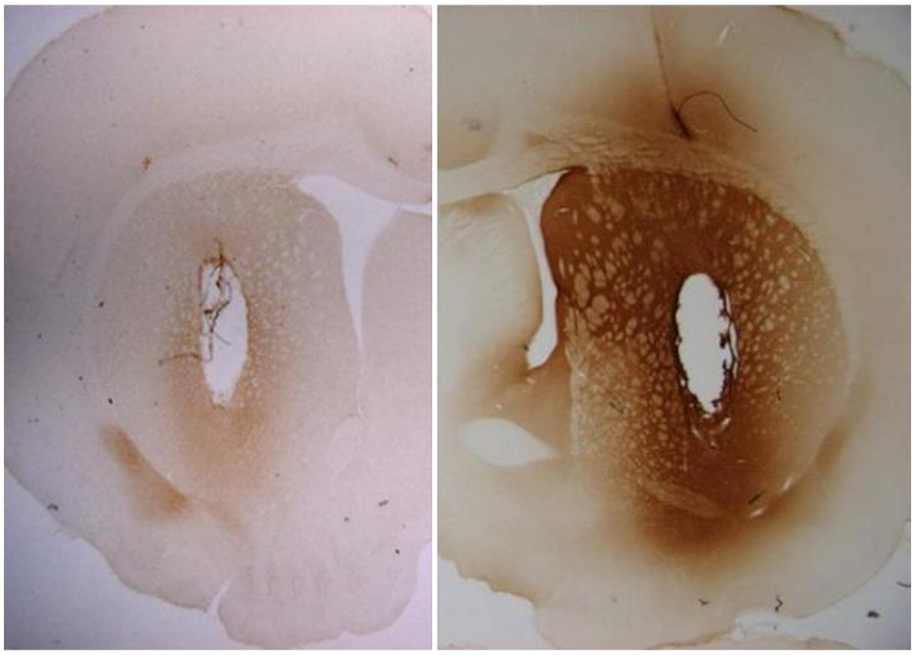

Fig. 2. Improvements in secretion of trophic factors from encapsulated cells enable significantly greater distribution of those proteins in the brain tissue. Rats were implanted intrastriatally with devices containing clonal human ARPE-19 cell lines producing GDNF. In the left panel, cells were modified to produce approximately 10 ng/day of GDNF which resulted in limited and low level diffusion of GDNF from the implant site. In contrast, cells generated using the Sleeping Beauty transposon system resulting in GDNF secretion approximately 10 times greater than that previously observed. The enhanced secretion is coupled with widespread distribution of GDNF throughout the implanted striatum.

therapeutic molecules to the brain. From its first conception proof of principle studies in animal models provided compelling reasons to believe that this approach could solve many of the problems that other approaches including direct brain infusion, gene therapy approaches, cell-based delivery, and biomaterial-based drug delivery were and continue to encounter. Cell encapsulation seemed capable of allowing site specific delivery of potent molecules for prolonged periods of time in a manner that was controllable and reversible. At the same time larger scale preclinical and clinical studies made it clear that these results were inconsistent enough to limit clinical development of this technology despite significant efforts in both academic laboratories and biotechnology companies. Recently, however, the development of "humanized" cell systems using human cells modified to secrete and deliver previously unachievable levels of trophic molecules have given birth to a new generation of technology capable of yielding a clinically viable platform technology for restorative and functional neurosurgery and the treatment of degenerative brain diseases. Ongoing studies in animal models and human clinical trials are now providing the type of data that was initially envisioned when cell encapsulation was originally pioneered. These studies may finally answer the questions of whether optimal delivery of factors such as GDNF to the brain are efficacious and whether these effects take the form of neuroprotection or (and) regeneration of CNS circuits.

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