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The Development of Encapsulated Cell Technologies as Therapies for Neurological and Sensory Diseases.

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Abstract

Cell encapsulation therapies involve the implantation of cells that secrete a therapeutic factor to provide clinical benefits. The transplanted cells are protected from immunorejection via encapsulation in a semipermeable membrane. This treatment strategy was originally investigated as a method for protecting pancreatic islets from immunorejection, thus allowing them to secrete insulin as a chronic treatment for diabetes. Since then a significant body of work has been conducted in developing cell encapsulation therapies to treat a variety of different diseases. Many of these conditions involve neurodegeneration, such as Alzheimer's and Parkinson's disease, as cell encapsulation therapies have proven to be particularly suitable for delivering therapeutics to the central nervous system. This is mainly because they offer chronic delivery of a therapeutic and can be implanted proximal to the affected tissue, bypassing the blood brain barrier, which is impermeable to many agents. Whilst these therapies are not yet widely available in the clinic, promising results have been obtained in several advanced clinical trials and further developmental work is currently underway. This review specifically examines the development of encapsulated cell therapies as treatments for neurological diseases and evaluates the challenges that are yet to be overcome before they can be made available for clinical use.

Keywords

Encapsulation, neurotrophin, Alzheimer's disease, Parkinson's disease, hearing loss, retinal degeneration, epilepsy, stroke

1.0 Introduction

Cell encapsulation therapy is the delivery of a therapeutic substance using cells encapsulated in a semipermeable membrane. It was originally investigated as a method for providing chronic insulin delivery to treat diabetes without the need for immunosuppression, using pancreatic islets encapsulated in a semipermeable membrane [1]. As a treatment for diabetes, cell encapsulation therapy represents a significant improvement over conventional treatments, such as repeated insulin injections and transplantation of unencapsulated islets. As encapsulated pancreatic islets are responsive to elevations in blood sugar levels, there is no need for repeated insulin injections. The islets are also protected from immunorejection by the encapsulation material, thus chronic immunosuppression, required following implantation of unencapsulated islets, is not necessary. The semipermeable encapsulation material is also permissive of the exchange of wastes and nutrients, thus facilitating the survival and function of the encapsulated islets over long periods post transplantation (figure 1). Thus, as a treatment for diabetes, cell encapsulation therapy represents a significant improvement over current therapies. These benefits are an example of the broader potential of cell encapsulation therapy as therapies for other chronic diseases, of which there are few or no effective treatment options.

Cell encapsulation therapies have also been developed as potential treatments for a variety of neurological diseases. One of the reasons for this broad applicability is that the encapsulated cells can be genetically manipulated to secrete practically any therapeutic protein that the gene sequence is known for. These therapies are particularly useful to deliver therapeutics that cannot be delivered systemically, such as neurotrophins, which elicit significant side effects when delivered systemically and have a short half-life [2, 3]. Neurotrophins are proteins that have significant survival effects on neurons and have demonstrated potential in supporting neuronal populations that degenerate in diseases such as Alzheimer's and Parkinson's

disease [4, 5]. Numerous neurological studies have demonstrated that cell encapsulation therapies are safe and efficacious in pre-clinical and clinical studies and clinical trials are currently underway for a number of cell encapsulation therapies for several neurodegenerative diseases. The first Phase I clinical trials to be conducted using cell encapsulation therapies for neurological disorders were completed in the mid 1990's in the context of amyotrophic lateral sclerosis and chronic pain but no further trials were conducted [6, 7]. However, other cell encapsulation therapies are currently in advanced clinical trials following promising results in preclinical and early clinical studies. This review deals with the application of encapsulated cell technologies to treat disorders of the peripheral and central nervous systems, as summarised in table 1. It reviews the progress made and the challenges yet to be resolved regarding the development of implants for clinical application.

2.0 Neurological Diseases

2.1 Parkinson's Disease

The underlying etiology of Parkinson's disease (PD) involves the loss of neurons in different regions of the brain, with most clinical emphasis focussed on the dramatic and disease-defining loss of dopaminergic neurons in the substantia nigra pars compacta. PD is characterized by motor deficits such as a resting tremor, rigidity, bradykinesia and altered posture, symptoms which are often followed later in the disease course by dementia [8, 9]. Much of the motor dysfunction associated with PD results from the loss of nigral dopamine projections to the striatum, but the cause of dementia is not clear [8]. Age is a major risk factor for PD, the incidence of PD in the fifth decade of life is 17.4 per 100,000 people, which increases to 93.1 per 100,000 people in the seventh decade of life, with a median onset of 60 years [10, 11]. Therefore, aging populations will see an increasing disease burden. Worldwide it is estimated that 4 million people are affected [12]. The total economic impact of PD is

difficult to estimate but in the USA alone the total annual figure could run as high as $US23 billion [12].

Current pharmacological treatment of PD usually involves oral administration of levodopa (L-DOPA), the precursor to dopamine, to replace what would normally be produced by lost dopaminergic neurons. The efficacy of this treatment is well established, especially in the early stages of PD [13]. However, chronic, systemic administration of L-DOPA results in undesirable side effects [14, 15] and over time the threshold L-DOPA concentration required to elicit side effects decreases, limiting the dosages that can be used safely and hence the effectiveness of the drug [16]. Cell transplantation has been investigated as a method to deliver a more continuous and physiologically 'normal' supply of dopamine to overcome the side effects of systemic L-DOPA administration. Adrenal chromaffin cells were initially used because they naturally produce neurotrophic factors and dopamine. Initial clinical studies using autografts of unencapsulated chromaffin cells demonstrated potential, but the results of several subsequent studies were unsatisfactory, partly due to poor cell survival but also due to a variety of surgical complications resulting in high morbidity [17-20].

Further experimental studies utilized chromaffin cells or PC12 cells, a pheochromocytoma cell line, encapsulated in hollow fibre poly(acrylonitrile-co-vinyl chloride) polymers or poly-l-lysine (PLL) coated alginate capsules [21-23]. In rat models of PD, these implants were effective in increasing the duration of efficacy of systemically-administered L-DOPA over a time course of weeks. However, in the context of PD this is a comparatively short time span and therefore further development is required to extend this timeframe to make these implants clinically relevant.

PD pathogenesis has also been linked to neurotrophin deficiencies in the brain and therefore the delivery of the neurotrophins such as brain-derived neurotrophic factor (BDNF) and glial cell-derived neurotrophic factor (GDNF) has been investigated as a treatment strategy. The delivery of both BDNF and GDNF to the brain via intrathecal and intracerebral injection and unencapsulated genetically modified cells has shown potential in supporting dopaminergic neurons and reducing Parkinsonian symptoms in animal models of PD [5, 24-30]. A Phase I clinical trial investigated GDNF delivery via mechanical pump intracerebroventricularly, however no improvements were observed and there was evidence of adverse side effects, such as nausea and depressive symptoms, resulting in the trial being halted in 2004 [31-33]. These negative outcomes may have been due to limited penetration of GDNF into the brain [31]. Two further Phase I trials were then conducted, which used cannulas to deliver GDNF directly to the putamen. Patients in the first of these studies demonstrated improvements in mobility and increases in tyrosine hydroxylase immunoreactivity, the rate-limiting enzyme in dopamine biosynthesis, and tyrosine hydroxylase-positive neurons were also observed in the substantia nigra of treated patients [34]. The second trial involved 34 patients, half receiving GDNF and half receiving a placebo. However, behavioural improvements were not observed in treated patients, despite increased dopamine uptake in the putamen [35]. It is possible that this increased uptake did not then lead to increased dopamine release from these neurons [35]. These trials demonstrate that the method and target of GDNF delivery is critically important in designing an effective PD treatment using neurotrophins and that delivery via cannula to the putamen or ventricles is not suitable.

As cell encapsulation devices can provide targeted, chronic delivery of neurotophins, they potentially represent a clinically-applicable neurotrophin delivery method. Several preclinical studies have been conducted using GDNF-secreting cells encapsulated in a polyvinyl alcohol matrix contained in poly(ether sulfone) hollow

fibers in both rat and baboon models of PD (figure 2) [36, 37]. These implants produced neurotrophins in the nanomolar range and, in rats, preserved dopaminergic neurons in the substantia nigra and were well tolerated [38-40]. In baboons, the implants required surgical replacement every 20 days and, despite multiple surgeries, implants were well tolerated with no noticeable inflammatory reaction at the sites of surgery [38]. This methodology, though impractical in a clinical setting, was successful in eliciting transient recovery of locomotor activity and increases in DOPA uptake, but not in protecting neurons from death. This may indicate that doses higher than the nanomolar range are required for neuroprotection in larger mammals. Whilst these preclinical studies have yielded promising results, these devices are yet to be tested in a clinical trial as a treatment for PD.

2.2 Stroke

A stroke is a localized area of brain infarction, which often results in permanent damage and loss of function. The two main types of stroke are ischemic stroke, due to blood vessel occlusion, and haemorrhagic stroke, caused by rupture of a blood vessel in the brain. Important risk factors for stroke include hypertension, diabetes, hyperlipidemia and tobacco smoke [41]. Stroke is the third leading cause of death and the leading cause of serious, long-term disability in the United States, approximately 795,000 people suffer a stroke annually in the United States and the total projected cost of stroke in 2009 was $68.9 billion [41].

Neurotrophins such as BDNF have demonstrated neuroprotective effects post stroke in animal models and could therefore potentially be used to preserve neurons post infarction [42, 43]. Devices consisting of cells transfected to secrete GDNF and encapsulated in polysulfone hollow fiber membranes have been tested in rats by implanting them into the brain prior to an ischemic insult [44]. This was successful in reducing neuronal damage caused by the insult [44]. Choroid plexus (CP) cells,

which secrete a variety of neuroprotective substances including BDNF, nerve growth factor (NGF), neurotrophin-3 (NT-3) and fibroblast growth factor (FGF), have also been used in the context of stroke [45]. CP cells, encapsulated in alginate microcapsules and implanted into the brain, showed protective effects against ischemic insults in rats [46, 47].

Glucagon-like peptide-1 (GLP-1) is another protein that exhibits neuroprotective and neurotrophic activity and has anti-apoptotic effects on neurons [48, 49]. GLP-1 has been tested successfully in animal models of traumatic brain injury, using devices consisting of stem cells transfected to secrete GLP-1 encapsulated in alginate microcapsules [49-51]. As yet this device has not been tested in clinical trials. Another device is also currently being trialled in a Phase I/II clinical trial sponsored by Cellmed/Biocompatibles [52]. This device consists of stem cells transfected to secrete CM1, a proprietary version of GLP-1, which is also anti-apoptotic [53]. It is designed to treat intracerebral haemorrhage, a severe form of stroke. As yet data has not been published from this trial.

2.3 Epilepsy

Epilepsy is one of the most common neurological disorders, affecting over 50 million people worldwide and accounting for 1% of the total global burden of disease [54]. Whilst not all causes of epilepsy are currently understood, any insult that disturbs neuronal function is an important risk factor, such as head trauma, genetic abnormalities, infection and tumours [55]. The economic impact of epilepsy is significant, estimated at $15.5 billion annually in the USA alone [56]. Up to 70% of patients with epilepsy can be successfully treated with anti-epileptic medication, however, these drugs carry with them the risk of adverse effects, including dizziness, sedation, impairment of cognitive function and potential teratogenic effects [57]. In 25 to 30% of patients, seizures are drug resistant and cannot be controlled by

medication [54]. In these patients, therapeutic options are surgery to remove the area of the brain where seizures originate or attempts to supress seizure activity via vagal nerve stimulation [57, 58].

Neurotrophins have been studied as potential therapies for epilepsy and whilst their therapeutic effects are clear in the context of neurodegenerative diseases such as PD, their benefits in the context of epilepsy have not been as evident. In animal models, neurotrophins have been shown to either diminish or worsen symptoms, depending on the dosage administered [59-63]. Larger doses of neurotrophins such as GDNF or BDNF have detrimental effects whilst the continual administration of smaller doses of neurotrophins is beneficial in reducing the symptoms epilepsy [60, 61]. Therefore, dosage is of critical importance. The chronic delivery of relatively smaller doses of neurotrophins has been achieved in animal models using implants consisting of cells transfected to secrete BDNF or GDNF encapsulated in polyethersulfone hollow fiber membranes, which are implanted into the brain [60, 61]. Promising results have been obtained in these animal models but as yet they have not been tested in clinical trials.

2.4 Huntington's Disease

Huntington's disease (HD) is a genetic neurodegenerative disease caused by the expression of a mutant form of the protein huntingtin which has deleterious effects on certain populations of neurons [64]. It is one of a group of diseases classified as polyglutamine diseases, which are caused by an expansion of CAG repeats in gene sequences, resulting in proteins that have an expanded stretch of glutamine in their amino acid sequence. Neurons of the striatum are particularly affected, although degeneration also occurs in the cortex and hippocampus and these losses also contribute to the pathogenesis of the disease [65-67]. HD is one of the more common genetic neurodegenerative disease, with a prevalence of 5-7 per 100,000 people

[68]. Typical duration from diagnosis of HD to death is 20 years, at which point motor and cognitive deficits are severe, and there are no treatments currently available [68]. However, unlike other neurodegenerative diseases, early detection is possible via genetic testing for the mutant gene, which is expressed in cells throughout the body [69]. Therefore, the ability to detect patients who harbour the mutant huntingtin gene long before symptoms become apparent provides a treatment window that could be exploited to provide support for affected neurons.

The capacity for neurotrophins to preserve populations of striatal neurons in rodent and non-human primate models of HD is well documented [70-79]. However, these studies used repeated intracranial injections, which is not a clinically viable treatment strategy. The use of cell-based therapy has been investigated as an alternative. This research has focused on two neurotrophic factors, NGF and ciliary neurotrophic factor (CNTF). The implants used in these studies consisted of calcium phosphate-transfected cells mixed with collagen and encapsulated in implants consisting of hollow fibers of poly(acrylonitrile-co-vinyl chloride). In rats and non-human primates, these implants showed protective effects on multiple populations of affected striatal neurons [73, 74, 80, 81]. In rats these implants have been shown to provide a sustained release of NGF for up to one year without adverse effects [80]. A Phase I clinical trial has also been performed using capsules loaded with cells transfected to secrete CNTF in six patients [82]. This study showed that the devices themselves were well tolerated and positive electrophysiological changes were observed in three patients, indicating improved neural circuit function [82]. However, variable survival of the encapsulated cells resulted in variable CNTF secretion [82]. As such, further optimisation of the encapsulation technology is required to achieve greater clinical efficacy. No new clinical trials have been initiated using these implants since publication of the Phase I trial results in 2004 [82].

Cells from the CP are another possible treatment for HD. In rats and non-human primates with striatal lesions, CP cells encapsulated in poly-ornithine coated alginate yielded significant increases in the volume of the striatum and performance in behavioural tests [83-86]. In both animal models only minor tissue reactions were reported and the implants were well tolerated. Further work and optimisation of these implants is required to achieve maximum clinical benefit but current work demonstrates their potential to at least slow the disease course of HD.

2.5 Alzheimer's Disease

Alzheimer's disease (AD) is the most common form of dementia in people over 60 and is characterised by a progressive loss of memory and cognition. The main risk factor of AD is age, incidence almost doubles every 5 years post 65 years of age [87, 88]. It is a complicated, multifactorial condition whose pathogenesis is incompletely understood. In 2006 the number of people worldwide with AD was 26.6 million and this figure is expected to quadruple by 2050 [89]. Worldwide, populations are aging and this in itself is likely to contribute greatly to increasing the incidence of AD. In 2009 in the USA alone, the annual cost of AD was estimated at US$172 billion and AD was cited as the seventh leading cause of death [90]. There are no completely effective treatments for AD and current clinical strategies involve treatments based on cognitive and neuropsychiatric symptoms of the disease [91]. Commonly used treatments are cholinesterase inhibitors to improve cognitive function and antipsychotic drugs to treat agitation and psychosis in AD patients with dementia [91].

In the brain, AD is characterized at the cellular level by the appearance of senile plaques and neurofibrillary tangles, which are aberrant accumulations of proteins that are associated with a significant loss of neurons and synapses in the brain [92]. In addition to abnormal protein accumulation, disturbances in neurotrophins in the brain

have also been linked to AD pathology. Neurotrophin receptors are normally expressed at high levels on neurons in the basal forebrain, but expression is drastically reduced in late-stage AD [93]. BDNF levels are also depressed in the AD brain and several studies have shown that decreases in BDNF are associated with AD pathology and that neurons containing neurofibrillary tangles do not contain BDNF [94, 95]. Studies in rodents and primates have shown that exogenous BDNF in the brain positively influences learning and memory, and can reverse cognitive decline and neuronal atrophy seen in these animal models of AD [96, 97]. Therefore neurotrophins show significant promise as a possible therapeutic for AD.

CNTF has been tested in a mouse model of AD using myoblasts transduced to secrete CNTF and encapsulated in alginate microcapsules [98]. When implanted intracerebroventricularly into mice expressing mutant amyloid precursor protein, or mice injected with amyloid beta, there were significant improvements in cognitive function [98]. GLP-1 has also been tested as a therapy for AD and has been shown to reduce amyloid deposition and has protective effects on neurons against toxicity induced by amyloid beta [48, 99]. To test this molecule in a cell encapsulation setting, human bone marrow-derived stem cells, transfected to secrete GLP-1, were encapsulated in alginate and implanted intracerebroventrically into a transgenic mouse model of AD [100]. In these animals, encapsulated GLP-1 secreting cells were effective in reducing amyloid deposition and suppressing the inflammatory response [100].

NGF has also shown significant therapeutic effects against AD. Studies in rodent and non-human primate models of AD have shown that NGF prevents retrograde degeneration of cholinergic neurons and can also correct spatial memory deficits [101-103]. A Phase I clinical trial in patients with mild AD was also conducted whereby autologous, unencapsulated grafts of fibroblasts transduced to secrete NGF

were implanted into the basal forebrain. No adverse effects were observed during this 22 month trial and there were indications of a decrease in the rate of cognitive decline [4]. Several studies have also utilized transfected NGF secreting cells encapsulated in asymmetric hollow fibers of poly(acrylonitrile-co-vinyl chloride) microspheres [80, 81, 104, 105]. In non-human primates, these implants provided support to degenerating neurons in the basal forebrain and promoted resprouting of cholinergic fibers [105, 106]. Implants were also well tolerated and only a minimal astrocytosis proximal to the implants was observed [81]. Whilst these are promising results, the time course of these experiments were approximately one month, which is short in the context of AD [81, 105]. However, in another study these microspheres were implanted into the ventricle of rats over a 13.5 month period; no adverse effects were observed and the microspheres were still capable of secreting NGF at the completion of the study [107]. Furthermore, robust sprouting of cholinergic fibers was observed proximal to the implant, indicating the concentrations of NGF secreted by these implants were sufficient to have trophic effects on surrounding neurons [107].

A Phase Ib clinical trial was conducted in 2008-2009, sponsored by NsGene, using encapsulated NGF-secreting cells (nsG0202) in six AD patients [108]. Four nsG0202 implants were implanted into the basal forebrain nuclei of each patient for a period of 12 months. Data from this trial is not yet published however the devices are reported to be well tolerated and there are promising indications of efficacy [109]. Positive results from this trial would potentially lead to multicentre clinical trials, thus moving this treatment closer to clinical availability.

2.6 Amyotrophic Lateral Sclerosis

Amyotrophic lateral sclerosis (ALS) is a debilitating, terminal condition characterized by a progressive loss of motor neurons leading to limb paralysis and eventually respiratory failure. It is a relatively rare condition, with an incidence of 1.5-2.5 per

100,000 people, but there is no cure and mean survival post onset of symptoms is three to five years [110]. Whilst the cause(s) of ALS remain unknown, approximately 10% of cases are dominantly inherited and 20% of these cases are due to mutations in the superoxide dismutase-1 gene [111].

Neurotrophins have been shown to provide neuroprotective effects against motor neuron degeneration and therefore represent a possible treatment [2, 112]. The majority of research has been performed using CNTF and promising results in animals led to a Phase I clinical trial involving systemic administration of CNTF [113]. However, as CNTF is rapidly cleared from the body, relatively large doses were required, which in turn resulted in unacceptable, often severe, side effects [2].

To overcome these adverse side effects, cell-based therapies were subsequently studied. In rats, implants consisting of a porous polypropylene filter containing cells transfected to secrete CNTF were capable of slowing axotomy-induced cell death of the facial nerve [114]. These implants were well tolerated and elicited only a small amount of fibrotic tissue growth around the capsules with no penetration of host cells [114]. In a murine model of motor neuronopathy, these implants were effective in increasing survival time by 40% and significantly decreasing motor neuron loss [115]. A similar implant using a hollow fiber membrane constructed from poly(ether sulfone) and containing myoblasts transfected to secrete CNTF was tested *in vivo* by implantation intrathecally in rats for 3 months [116]. These implants were capable of secreting CNTF for the 3 month implantation period and provided some rescue effect on axotomy-induced neuronal death [116]. A Phase I clinical trial then followed in which six patients were implanted intrathecally for three months, during which time the implants significantly increased CNTF levels in the cerebrospinal fluid (CSF) without the side effects associated with systemic delivery [6, 7]. These implants were also very well tolerated as there was no evidence of cells adherent on the implants

following their removal at the conclusion of the study [6]. However, it was unclear as to whether disease progression was slowed by the implants, thus necessitating further optimization of this strategy to yield clinical benefit and as yet no new clinical trials have been undertaken since the publication of these results in 1996 [6].

In addition to CNTF, GDNF and vascular endothelial growth factor (VEGF) have also demonstrated therapeutic potential in superoxide dismutase-1 (SOD-1) mutant rats and mice, which are models of ALS. Autologous myoblasts or bone marrow-derived mesenchymal stem cells were transduced to secrete GDNF and implanted intramuscularly into SOD-1 mutant rats and mice prior to disease onset [117, 118]. This therapy increased motor neuron survival, delayed disease progression and increased lifespan [117, 118]. VEGF has also been shown to prevent motor neuron degeneration and prolong survival of SOD-1 mutant rodents when delivered intraperitoneally or intracerebroventricularly [119-121]. Two Phase I/II clinical trials sponsored by NeuroNova are currently underway to test the efficacy of VEGF administration via a pump and catheter system intracerebroventricularly [122, 123]. Promising results from this clinical trial could potentially lead to the development of cell encapsulation therapies to deliver VEGF, bypassing issues inherent with a pump-based catheter system.

2.7 Chronic Pain

Chronic pain is a serious medical problem for a significant number of patients who cannot achieve adequate relief. Whilst an accurate definition is somewhat controversial, it can be defined as pain that extends beyond the expected time frame of healing. Chronic pain affects at least 116 million adults in the USA alone at a cost of $560-635 billion annually [124]. Treatment of chronic pain commonly involves systemic delivery of opioids but there are significant issues associated with these drugs, especially when used over long periods of time. Insensitivity to their actions

386 can result, necessitating increased dosages that results in further desensitisation and
387 increased likelihood of adverse reactions and side effects, such as cognitive
388 impairment, chronic constipation and respiratory depression. With increasing dosage,
389 side effects can eventually reach a stage where they become unmanageable or
390 unacceptable to the patient, negating any beneficial effects of the drug. The
391 production and use of opioids also places a significant strain on health care systems
392 [125, 126].
393
394 A more 'natural' treatment for chronic pain involves utilizing adrenal chromaffin cells,
395 which secrete a number of anti-nociceptive substances, such as catecholamines,
396 adrenaline, nor-adrenaline, opioid peptides, met-enkephalin and leu-enkephalin [127,
397 128]. As these substances are naturally secreted by chromaffin cells, they are not
398 foreign to the body and therefore pose less risk of side effects and adverse reactions
399 than opioids [127]. Chromaffin cells also express nicotinic receptors, which stimulate
400 secretion of these substances when activated by nicotine, which is a feature that
401 could be utilized *in vivo* to achieve a level of control over release [129].
402
403 There are numerous studies investigating the potential of encapsulated chromaffin
404 cell implants to treat chronic pain, mainly in rat models of pain. Early studies using
405 suspensions of bovine chromaffin cells injected intrathecally demonstrated promising
406 results in alleviating chronic pain [130-132]. Subsequent studies used bovine
407 chromaffin cells and PC12 cells, a pheochromocytoma cell line, encapsulated in PLL
408 coated alginate capsules. In these studies, encapsulated cells were implanted
409 intrathecally in rats and, in treated animals, levels of norepinephrine and met
410 enkephalin were significantly increased in the CSF in response to pain, indicating an
411 antinociceptive effect [133-136].

A Phase I clinical trial was conducted with a cohort of patients that were experiencing inadequately managed chronic pain. Patients received implants consisting of bovine chromaffin cells in alginate contained in poly(acrylonitrile-co-vinyl chloride) (PAN/PVC) semipermeable membranes. The implants were well tolerated and there was no evidence of tissue or cellular growth on the surface of the capsules. This study described improvements in the pain ratings reported by implant recipients but did not control for placebo effects [7]. Results from this trial were published in 1996 and as yet no new trials have been initiated [7]. A Phase II clinical trial was also conducted, which was a longitudinal study of 15 patients with intractable cancer pain that were implanted with unencapsulated human adrenal medullary tissue intrathecally. This treatment strategy was safe and effective but one of the main disadvantages of the procedure was the requirement for immunosuppression, which could be overcome by encapsulating the adrenal tissue [137]. Whilst further work is required, these treatment strategies are potentially clinically viable and would solve many of the issues surrounding chronic opioid use, especially those related to desensitisation and side effects.

3.0 Sensory Diseases

3.1 Hearing Loss

Hearing loss reduces the capacity for communication, which can have a major impact on the ability to obtain employment, participate in education and gain skills, and engage in social relationships. Hearing loss also has a significant impact on the health care system. In developed countries, rates of hearing loss are approximately 17% of the adult population (36 million people in the USA). However this figure is very dependent on age and is as high as 47% in adults 75 years old and over in the USA. The economic impact of hearing loss in the USA is in excess of $100 billion annually [138].

The most common form of hearing loss is sensorineural hearing loss (SNHL), which typically occurs following damage to, or loss of, cochlear hair cells - the receptors responsible for converting the mechanical vibrations of sound into nerve impulses in auditory neurons (ANs). Widespread hair cell loss results in severe to profound SNHL and the only effective therapeutic intervention for these patients is the use of a cochlear implant, a neural prosthesis designed to electrically stimulate the auditory nerve in order to provide the pitch and temporal cues necessary for speech perception. However, ANs undergo progressive degeneration in the absence of hair cells, ultimately resulting in significant neuronal loss after long periods of deafness [139, 140]. Experimental studies from our laboratory indicate that ongoing AN degeneration can compromise the efficacy of the cochlear implant, therefore, there are likely to be important clinical benefits in rescuing ANs from degeneration [139, 141-143]. The loss of endogenous neurotrophic factors, such as BDNF and NT-3, normally expressed by hair- and support-cells within the organ of Corti, initiates AN degeneration [144-147]. Numerous studies have demonstrated that intracochlear administration of these neurotrophins via a mini-osmotic pump and cannula-based system can support AN survival in animal models of deafness [148-151]. When combined with chronic electrical stimulation via a cochlear implant, exogenous neurotrophin treatment results in significantly enhanced AN survival compared to neurotrophin treatment alone [150, 152].

Whilst these studies have shown the benefits of using neurotrophin delivery combined with electrical stimulation, the delivery of neurotrophins via a mini-osmotic pump/cannulae assembly is not acceptable as a therapy for preserving hearing in a clinical setting. This is due to the finite capacity of the pumps, which necessitate refilling for long-term use, and concerns about infection with multiple use of a cannula or manipulation of an osmotic pump. Therefore, cell encapsulation technology presents an attractive alternative technique as they can be implanted along with the

cochlear implant as part of a once-off surgical procedure and provide the potential for long-term delivery of neurotrophins. Experiments in our laboratory have shown that Schwann cells genetically modified to secrete BDNF or NT-3 are able to enhance the survival of ANs *in vitro* [153]. The AN survival-promoting effects of BDNF-secreting Schwann cells were subsequently tested *in vivo* by encapsulating them in PLL coated alginate capsules prior to implantation into deafened guinea pig cochleae (figure 3) [154]. The implants were generally well tolerated and did not cause an adverse reaction. Importantly, in comparison to control (empty) capsules, the implantation of encapsulated BDNF-Schwann cells enhanced AN survival [154]. Similar results were also obtained in cats using CP cells encapsulated in PLL coated alginate [155]. In combination with electrical stimulation from a cochlear implant, this therapy was effective in supporting AN survival in neonatally deafened cats for periods of at least 8 months [155].

Another cell encapsulation technique that has undergone preclinical evaluation consists of a cochlear implant incorporating an electrode array coated in an agarose gel containing BDNF secreting cells [156]. Over a 48 day trial *in vivo*, the implant was effective in supporting ANs and elicited only a minimal tissue reaction. However, the exchange of wastes and nutrients was not sufficient to support the cells for any significant length of time, suggesting that an alternative material would be more suitable for this application [156]. Moreover, there is the potential to extend this technology to target the rescue of cochlear hair cells.

Studies to date have shown that the implantation of encapsulated cells into the cochlea along with a cochlear electrode array is achievable and therefore potentially clinically viable. However, further data is needed, particularly regarding the long-term safety and performance of implants in preclinical studies and clinical trials. However, neurotrophin delivery to ANs using encapsulated cells in combination with chronic

electrical stimulation from the cochlear implant shows significant potential as a treatment to provide functional benefits for cochlear implant patients.

3.2 Vision Loss

Diseases that result in the degeneration of the retina, producing progressive loss of peripheral vision and eventually central vision loss and blindness, are a significant public health problem. In the USA alone the estimated cost of vision impairment has been estimated at $35.4 billion [157]. The two most common conditions involving retinal degeneration are retinitis pigmentosa (RP) and age-related macular degeneration (AMD) [158]. RP is characterized by the death of photoreceptors in the periphery of the retina and has complicated and diverse genetic origins that are increasingly being understood [159]. The cause of AMD is even less clear but has origins in the accumulation of waste products in the macula (dry AMD) or the formation of abnormal blood vessels in the retina that allow the leakage of blood and fluid, resulting in swelling and vision impairment (wet AMD) [160, 161]. Like RP, AMD is characterised by a loss of photoreceptors, which particularly affects central vision, that then sets in place additional degenerative changes in the retina [162].

Treatments for these conditions are limited and currently there are no specific treatments for RP or dry AMD [163, 164]. However, a relatively new treatment for wet AMD is available, which involves intraviteal injections of an anti- VEGF antibody or the antigen binding fragment of the same antibody [165]. VEGF is a major factor associated with the formation of new blood vessels in wet AMD and therefore this treatment acts to inhibit their formation. Whilst anti-VEGF treatments are effective in improving visual acuity, repeated intraocular injections carry the risk of bacterial infection which represents a significant risk to vision. However, this has been documented in only 1% of cases in a clinical trial [166-168].

Studies into potential treatments for dry AMD and RP have shown that injection of neurotrophins such as FGF and CNTF into the eye provide protection against retinal photoreceptor degeneration [169-171]. In addition, several neurotrophins exert protective effects on neurons in inner retinal layers, CNTF being one of the most effective in this setting [172]. This is important because retinal ganglion cell (RGC) loss can follow degeneration of photoreceptors in the outer retina [162, 173, 174], presumably associated with a loss of trophic support in a manner similar to the loss of ANs following the degeneration of hair cells in the cochlea.

Whilst intraviteal injections of neurotrophins support the survival of cell populations in the eye, this strategy is not practical for long-term clinical applications [175, 176]. To overcome the need for repeated injections, strategies to achieve chronic delivery have been developed using encapsulated neurotrophin secreting cells, which have been tested in various animal models of RP. The anatomy of the eye makes it particularly suited to such treatment as it is a relatively contained environment and therefore secreted neurotrophins will be somewhat concentrated where they are most needed. These implants consist of CNTF secreting cells in a hollow fiber membrane consisting of poly(ethersulfone) containing an internal scaffold of poly(ethylene terephthalate) yarn, which promotes cell attachment [177, 178]. These implants were tested in rats, dogs and rabbits and were effective in protecting photoreceptors from degeneration and were well tolerated [178, 179]. A study in rabbits showed that this implant is capable of continuous delivery of CNTF at concentrations above therapeutic thresholds for up to one year [179].

Following these successful trials in animals, a Phase I clinical trial of six months duration was conducted to assess the safety and efficacy of these implants. This study demonstrated that implants recovered from patients still secreted CNTF at concentrations above those deemed to be therapeutic [180]. The implants were also

well tolerated, with no systemic or ocular complications observed, with the exception of a single choroidal detachment, which was deemed likely due to mechanical insults sustained during surgery [180]. There were also indications that visual acuity was improved in some patients, but interpretation of these results was hampered by variability, a small sample size of ten patients and lack of adequate controls. Longer term Phase II and a Phase II/III clinical trial are currently underway. A Phase II study, sponsored by Neurotech Pharmaceuticals, was designed to assess the safety and efficacy of their CNTF-producing NT-501 implant in patients with dry AMD over an 18 month follow-up period [181]. The NT-501 implant was also tested in a Phase II/III trial in patients with RP, which aimed to assess the performance of these implants in patients out to 2.5 years post implantation [182]. As yet no data has been published from these studies [177].

4.0 Future Directions and Conclusions

Significant progress has been made in the development of cell encapsulation therapies as treatments for neurological conditions. However, further challenges still exist before these therapies can be accepted into the clinic. Importantly, more data is needed regarding the longevity of cell encapsulation therapies, as these are designed to be chronic delivery methods. Of primary concern is that the implants are safe, i.e., they can remain in the host for long periods of time without causing adverse reactions. This necessitates that the encapsulation material must be stable *in vivo* for extended periods, thus remaining biocompatible and protecting the encapsulated tissue from immunorejection. Another important consideration is the consistency of the encapsulation material produced using scaled-up manufacturing techniques, which are required to produce sufficient numbers of devices for large scale clinical trials or for clinical use. Consistency is very important in gaining regulatory approval for use in clinical trials or in the clinic, as variations in the composition or purity of the materials could potentially lead to devices that fail *in vivo*.

This is particularly pertinent for alginate, as it is derived from algae, a natural product that can contain high levels of contaminating proteins. If adequate purification is not achieved, biocompatibility could be compromised, resulting in a foreign body reaction post implantation and possible capsule destruction [183, 184]. However, using current purification methods, millions of alginate capsules can be produced consistently under good manufacturing practise standards. Additionally, newer manufacturing technologies being developed could see the number of capsules able to be produced increase tenfold. Therefore, alginate is considered a viable material for large scale cell encapsulation therapy. Batch to batch variability is less of an issue for other materials, such as cellulose sulphate, which has been used successfully as part of a cell encapsulation therapy for pancreatic cancer in a Phase I/II clinical trial [185, 186]. Cellulose sulphate can now be produced in large quantities under good manufacturing practice, which is compatible with clinical use [187, 188].

Longevity data is also important in the context of the encapsulated tissue. Encapsulated cells must not proliferate within the encapsulation device to such a degree that they compromise the integrity of the device, which could potentially expose them to the immune system. The encapsulated cells must also be capable of secreting therapeutics for an acceptable period of time, depending on the therapy in question. Whilst there are still issues to resolve and more data to obtain, cell encapsulation represents a promising treatment strategy against a number of chronic diseases with limited or no treatment options currently available. Considering the social and economic impact of these diseases, the scope of potential benefits to be obtained from cell encapsulation therapies is large.

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Figure captions

Figure 1. General structure of a cell encapsulation device. Therapeutic-secreting cells are encapsulated in a biocompatible, semipermeable membrane that allows the release of therapeutics, such as neurotrophins, whilst excluding the immune system, preventing immunorejection. The membrane is also permeable to oxygen, nutrients and waste products, thus supporting the survival of encapsulated cells.

Figure 2. Polyethersulfone hollow fibers containing a polyvinyl alcohol matrix used to encapsulate GDNF-secreting human fibroblasts for implantation into the striatum. (a)–(d) Scanning electron micrograph images of the implant, (a); the glued-end (b); the hollow-fibre membrane pores (c,d); a high power cross-section, (e) a photomicrograph of encapsulated cells implanted for one month in the rat striatum. Devices of similar configurations have also been used the development of treatments for Huntington's and Alzheimer's disease [189].

Figure 3. Alginate microcapsules containing BDNF-secreting Schwann cells. Schwann cell clumps are visible within the capsule walls. Scale bar = 500μM.

Table 1

Disease	Device	Therapeutic	Stage of development	References
Parkinson's disease	Transfected mouse myoblasts in a polyvinyl alcohol matrix encapsulated in polyethersulfone hollow fibers	Neurotrophins (GDNF)	Preclinical (completed –published 2004)	[36-40]
Stroke	Stem cells transfected to secrete a modified GLP-1 protein encapsulated in alginate microcapsules	Neurotrophins (GDNF)	Phase I/II (ongoing)	[52]
Epilepsy	Human cell line transfected to secrete BDNF or GDNF encapsulated in polyethersulfone hollow fiber membranes	Neurotrophins (GDNF)	Preclinical (completed – published 2009 and 2011)	[60, 61]
Huntington's disease	Transfected baby hamster kidney cells in a collagen matrix encapsulated in hollow fibers of poly(acrylonitrile-co-vinyl chloride)	Neurotrophins (CNTF)	Phase I clinical trial (completed – published 2004)	[82]
Alzheimer's disease	Transfected baby hamster kidney cells in hollow fibers of poly(acrylonitrile/vinyl chloride) and poly(D,L-lactide-*co*-glycolide) biodegradable microspheres	Neurotrophins (NGF)	Phase Ib clinical trial (completed 2009 - not yet published)	[108]
Amyotrophic lateral sclerosis	Transfected baby hamster kidney cells in a porous polypropylene filter	Neurotrophins (CNTF)	Phase I clinical trial (completed – published 1996)	[6]
Chronic pain	Bovine chromaffin cells in an alginate matrix encased in a semipermeable membrane	Neuroactive, antinociceptive substances	Phase I clinical trial (completed – published 1996)	[7]
Hearing loss	Transfected schwann cells in poly-ornithine-coated alginate microcapsules	Neurotrophins and growth factors	Preclinical (completed – published 2011)	[154, 155]
Vision loss (age-related macular degeneration & retinitis pigmentosa)	Human retinal pigment epithelium cells in a poly(ethylene terephthalate) yarn scaffold encased in a semipermeable polysulfone hollow-fiber membrane	Neurotrophins (CNTF)	Phase II clinical trial (retinitis pigmentosa. Completed – not yet published). Phase II/III clinical trial (age-related macular degeneration. Completed – not yet published)	[181, 182]

Table 1. Summary of cell encapsulation devices used to treat various conditions described in this review and the most advanced stage of development each device is at currently. GDNF - glial cell-derived neurotrophic factor, CNTF - ciliary neurotrophic factor, NGF - nerve growth factor.