ELSEVIER

Advanced Drug Delivery Reviews 42 (2000) 81–102

advanced drug delivery reviews

www.elsevier.com/locate/drugdeliv

Cell delivery to the central nervous system

Molly S. Shoichet[a,*], Shelley R. Winn[b]

[a]*Department of Chemical Engineering and Applied Chemistry, Department of Chemistry, Institute of Biomaterials and Biomedical Engineering, University of Toronto, 200 College Street, Toronto, Ontario M55 3E5, Canada*

[b]*Department of Surgery, Oregon Health Sciences University, L352A, 3181 SW Sam Jackson Park Rd, Portland, OR 97201, USA*

Received 1 November 1999; accepted 14 January 2000

Abstract

A dysfunctional central nervous system (CNS) resulting from neurological disorders and diseases impacts all of humanity. The outcome presents a staggering health care issue with a tremendous potential for developing interventive therapies. The delivery of therapeutic molecules to the CNS has been hampered by the presence of the blood–brain barrier (BBB). To circumvent this barrier, putative therapeutic molecules have been delivered to the CNS by such methods as pumps/osmotic pumps, osmotic opening of the BBB, sustained polymer release systems and cell delivery via site-specific transplantation of cells. This review presents an overview of some of the CNS delivery technologies with special emphasis on transplantation of cells with and without the use of polymer encapsulation technology.

Contents

*Corresponding author. Tel.: +1-416-978-1460; fax: +1-416-978-8605.
E-mail address: molly@ecf.utoronto.ca (M.S. Shoichet).

0169-409X/00/$ – see front matter
PII: S0169-409X(00)00055-7

1. Introduction

Neurological disorders and diseases affect our everyday life, impacting nervous systems responsible for memory, cognition, language and voluntary movement. Some of the numerous disorders associated with a dysfunctional central nervous system (CNS) include Alzheimer's disease (AD) and other dementias, cerebrovascular disease (associated with stroke), epilepsy, Parkinson's disease (PD), multiple sclerosis (MS), spinal cord injury (SCI), dystonias, dysfunctions associated with head trauma and cancer, and psychiatric disorders. Other neurological deficits include chronic pain, Huntington's disease (HD), and Lou Gehrig's disease (or amyotrophic lateral sclerosis, ALS). It is estimated that these diseases/disorders affect more than 20 million people in the United States alone, accounting for over $400 billion annually for their treatment and prolonged care [1,2]. While care for individuals with neurological disorders has improved, the quality of life generally has not, largely due to the paucity of effective therapeutic treatments. This can be especially distressing for individuals with such neurological disorders as Epilepsy, MS, or SCI, who are chronically disabled at the prime of their life. In this paper we present some of the methodologies utilized to deliver neuroactive molecules to the CNS, with special emphasis on transplantation of cells with and without the use of polymer encapsulation technology. A thorough presentation outlining the recent advances in neurobiology was recently reviewed in a special issue on neurological disorders published in Nature [3].

Our limited understanding of their pathogenic mechanisms complicates developing treatments for many of these neurological disorders; however, significant progress has been made in the recent past that will allow new therapeutic approaches to be designed, tested and ultimately used for patient care. As the role of genetic factors continues to be elucidated, the likelihood for direct gene therapy interventions are inevitable, although the time course for successful application of this technology is years away. In the meantime, however, advances in protein and peptide chemistry are providing a vast array of neuroactive compounds having therapeutic potential as neuroprotective treatments [4]. The ability to deliver these molecules systemically is hampered by degradation and metabolism, either by relative short half lives of the molecules themselves, or metabolic degradation by the liver prior to reaching the CNS as a target site. In addition, the blood–brain barrier (BBB) — a protective cellular barrier that regulates the internal environment with a mechanism of low passive permeability combined with a highly selective transport system [5–7] — poses a significant obstacle for the delivery of many neuroactive factors. A number of techniques are available to permit delivery of molecules to the CNS that bypass the BBB and these are presented in Section 2.

One approach of overcoming the BBB that continues to be investigated is localized cell therapy. In contrast to traditional drug therapy, where a specific molecule is targeted for administration to the entire brain, cell therapy depends upon the de novo synthesis of one or several therapeutic molecules to be released by the cells into the tissue, or 'target' site, of the CNS. Two primary methods of cell therapy include transplants for cell replacement (nonencapsulated) and polymer-encapsulated cell therapy. In traditional cell replacement, the missing neurons or non-neuronal cells are replaced with cell transplants, providing the appropriate missing neurotransmitter. For example, in experimental PD, dopamine-rich neural grafts replenish the diseased or missing nigrostriatal dopamine neurons, establishing functional reinnervation to restore dopamine neurotransmission in the area surrounding the transplant of the denervated striatum [8]. Polymer-encapsulated

therapy provides a local source for the missing neurotransmitter, but the polymer barrier prevents cell-to-cell interactions, hence, no functional reinnervation is feasible. The advantages and disadvantages of cell therapy relative to osmotic pumps and sustained-release polymer systems are summarized in Table 1. Cell therapy strategies have primarily been targeted for PD [9–14], AD [15–19], HD [20–24], ALS [25], MS [26,27] and chronic pain [28–31].

In this paper, we discuss some of the delivery methods utilized to deliver neuroactive compounds to the CNS. Additionally, some of the key findings for both traditional cell replacement and encapsulated cell therapies are summarized (e.g. Table 2), with a view to distinguishing their advantages and disadvantages. Section 3 presents an overview of therapies directed at Parkinson's disease, including a presentation of the two cell therapy techniques, and issues related to transplant biology. For more detailed information on the methods and characterization of encapsulation technology, please see Chapter 5, Animal Cell Encapsulation.

2. Delivery methods

2.1. Overview

A number of strategies have been developed to circumvent the selective BBB. Some of the techniques currently available for neuroactive factor delivery to the brain include: (1) carrier-, or receptor-mediated transcytosis [32,33]; (2) osmotic opening [34,35]; (3) direct infusion with stereotactic guidance [36–38]; (4) osmotic pumps [39,40]; (5) sustained-release polymer systems [41,42]; (6) cell replacement/cell therapy [9–14,17,23–25,43–47] and (7) direct gene therapy [48–51]. This section highlights some of the advantages and limitations of these technologies.

2.2. Carrier-mediated transcytosis

The carrier- and/or receptor-mediated transport mechanisms at the blood–brain interface have been described for several endogenous peptides and pro-

Table 1
Some advantages and disadvantages of implantable delivery systems

Pumps	Controlled release	Cell therapy
	Advantages	
Quick delivery of therapeutic	Good short term release	Cells constituitively produce active therapeutics
Retrievable	Retrievable[a]	Retrievable[b]
Dosage can be regulated	Single, minimally invasive surgical procedure	Minimally invasive surgery
	Biocompatible	Biostable, biocompatible[c]
		Xenogeneic or engineered cells may be used without immunosuppression[c]
	Disadvantages	
Therapeutic may degrade in reservoir	Therapeutic may degrade	Potentially inadequate long term cell viability
Prolonged delivery may be limited	Dosage may be difficult to control[d]	Potentially difficult to regulate cell output
		Complex regulatory issues

[a] When biostable polymers used.
[b] When encapsulated cells used in macrocapsule geometry.
[c] For encapsulated cell therapy only.
[d] When biodegradable polymers used.

teins [52–54]. However, several physiologically important neuropeptides, e.g. nerve growth factors, lack abundant transporters on the microvascular endothelium of the BBB and are therefore not translocated across the BBB. To circumvent this deficiency, investigators have exploited endogenous cerebrovascular transport systems to deliver molecules of interest from the bloodstream to the brain: i.e. adsorptive endocytosis of specific cell surface oligosaccharides [55] or the receptor for transferrin (ferrotransferrin) [56,57].

In contrast to invasive neurosurgical techniques, neurotrophic factor conjugation to an antibody against the transferrin receptor provides a noninvasive delivery vehicle to the brain. Friden and colleagues [32,58] have shown transport across the BBB with an anti-rat transferrin receptor antibody, OX-26, linked to nerve growth factor (NGF). Kordower et al. [59], has shown that the OX-26-NGF conjugate is effective in preventing the degeneration of cholinergic striatal neurons in a rat model of Huntington's disease. However, a number of limitations of the technology persist. As is the case for many systemically delivered molecules, high systemic doses are required to reach significant levels in the CNS, and moreover, the effect is global and therefore does not permit localized brain delivery. Additionally, the receptor antibodies are species specific and chronic use of antibody conjugates, even humanized antibody, may stimulate an immune response.

2.3. Osmotic opening

Another means of delivering elevated levels of therapeutic molecules to the brain involves the transient osmotic disruption of the BBB endothelium [35,60]. This technology effectively increases the concentrations of chemotherapeutic agents in the brain and the cerebrospinal fluid of both preclinical brain tumor model systems [61] and clinical situations [35]. BBB disruption is designed to maximize the CNS drug delivery of chemotherapeutic agents. Drug delivery of these agents in conjunction with osmotic opening of the BBB can be increased by 50- to 100-fold. In a patient population of CNS lymphoma, neuropsychological follow-ups have indicated that cognitive functions were preserved and significantly improved in those patients receiving an initial BBB disruptive chemotherapy as compared to aged-matched patients receiving radiation therapy [62,63]. A plethora of preclinical models, as well as Phase 1 clinical trials, have been characterized over the past 20 years with a number of candidate molecules intended to outwit the BBB. However, until rigorous Phase III randomized studies are performed, with and without the use of BBB disruptive agents, the clinical impact and utility of the technology will remain in question.

2.4. Direct injection (microinjection)

Direct injections of molecules have been utilized for many experimental paradigms. For example, generation of animal models of degenerative disorders, such as PD and HD, can be accomplished by the direct injection of neurotoxins into specific locations in the host brain. The neurotoxins 6-hydroxydopamine and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) have been administered to create lesions of the nigrostriatal pathway to mimic the pathology of dopamine depletion in PD. Similarly for HD, striatal injections of excitotoxins such as kainic acid, ibotenic, or quinolinic acid produce a profile of neurochemical and pathological alterations very similar to that observed in the striatum of HD patients [13,14].

Single injections are a powerful tool to evaluate the diffusion pattern of the injected molecule. A single unilateral intraventricular injection of ^{125}I-NGF exhibited a bilateral distribution into septal cells, the hypothalamus and cerebellum [64]. However, the penetration into the host tissue was limited and combined with the observation of rapid degradation, the authors suggested that this method of delivery may be inadequate for neurotrophins. Similarly, Yan et al. [65] evaluated the brain distribution of NGF, BDNF and NT-3 after a single intraventricular injection. The distribution of these neurotrophins appeared to correlate with *trk* receptor expression; BDNF diffusion appeared limited to the ependymal cell layer of the ventricle, the cells of which express high levels of the *trkB* receptor that binds BDNF. NGF exhibited a more extensive pattern of distribution, while NT-3 distribution was intermediate between BDNF and NGF. A single

injection of NGF, BDNF and NT-3 into the ventricle and tissue striatal parenchyma was compared to a sustained delivery method via osmotic minipumps [66]. The observations described following the single injection paradigm paralleled those of Yan et al. [65] and the neurotrophin distribution and depth of penetration was generally determined to be concentration dependent.

An intraocular approach has been a valuable model to evaluate a site-specific effect of trophic factors and neurotrophins on transplants in a controlled environment [67,68]. Discrete transplants of the peripheral and central nervous system become well vascularized and develop a functional BBB [67,69]. Direct injections of NGF has been demonstrated to stimulate the growth of intraocular brain tissue transplants [70] and provides an isolated, well-defined model system to compare other methods of neurotrophin delivery, e.g., intravenous administration of an OX-26-NGF conjugate [71]. Direct and repeated injections are essential for labile substances and the most straightforward approach to bypass the BBB to deliver a therapeutic dose within a discrete site of the CNS. However, repeated injections can elicit additional injury to surrounding tissues and increase the risk for infection.

2.5. Pumps

Direct intra-CNS delivery of neuroactive molecules via pumps has been the preferred method for many experimental paradigms and has been successfully applied in several clinical conditions. Pumps offer a point source delivery of neuroactive agents that can quickly reach a sustainable therapeutic concentration. Pump technology is especially attractive for molecules that have systemic or peripheral actions resulting in negative side effects, or stable drugs that are effective with limited penetration into the tissue parenchyma of the brain [72]. Pumps can be implanted in the subcutaneous tissue with a drug reservoir. Drugs are delivered from the reservoir via a silicone tube and cannula placement leads to either the lateral ventricle or tissue parenchyma of the brain, or the epidural/intrathecal space of the spine.

Pump technology has evolved from the Ommaya® drug reservoir and Infusaid® pump to the more sophisticated Alzet® mini-osmotic pumps, the Medtronic pump, e.g., SyncroMed® and electronic infusion pumps. The limitations of the Ommaya reservoir and Infusaid pump are related to their requirement for the application of pressure to drive the delivery of drugs, resulting in inconsistent drug delivery [73,74]. Mini-osmotic pumps rely on osmotic pressure driven systems to achieve a steady rate of drug delivery. Electronic infusion pumps can maintain steady drug delivery for several years and can be easily reprogrammed outside the body.

The limited capability for the CNS to regenerate itself appears to be influenced by the presence of neurite growth-inhibiting molecules [75] and insufficient neurite growth-promoting factors [76]. Pumps have provided researchers with the ability to deliver some of the neurite growth-promoting factors, neurotrophins, in a sustained manner to the cerebrospinal fluid of the ventricle or spinal cord, as well as, brain tissue parenchyma. Local continuous infusions of neurotrophic factors, such as glial cell line-derived neurotrophic factor (GDNF) [77] is at least equivalent to intermittent intracerebral injections [78] in preventing experimental-induced death of dopamine neurons. GDNF exhibited equivalent levels of survival-promoting effects to some of the other neurotrophins, such as CNTF, NT-3, and NT-4 [79,80], and was more potent than BDNF [81]. Neurotrophic molecules have also been delivered via intraspinal infusions to promote regeneration of sensory axons in spinal cord models [82].

Implantable pumps to deliver intrathecal or epidural morphine or morphine substitutes are commonly used to deliver opiate medication for pain management in patients with terminal cancer [83,84]. In a recent retrospective study, 4–10% failures were associated with catheter dislodgement, 1–10% associated with leakage, and less than 1% with obstruction [84]. The ability to deliver pain relieving molecules in a sustained, site-specific manner has improved the quality of life for patients suffering from chronic pain.

The advantages of using osmotic mini-pump drug delivery include a minimal amount of tissue damage at the site of cannula placement. The drug is also administered with no intervention from the investigator or interaction with the animal once the pump is implanted. Hence, treatments can be performed on freely moving animals, an important consideration

for studies requiring behavioral monitoring. Also, a steady state concentration of drug can be delivered. However, the continuous nature of a mini-pump infusion is in some cases not desirable. For example, several neuronal and neuroendocrine systems appear to be regulated in a pulsatile fashion related to circadian-type rhythms.

The limitations to mini-pump infusions must be considered carefully. For many biomolecules, like the catecholamines and some of the neurotrophins, stability is a major concern. Glial cell line-derived neurotrophic factor exhibits approximately 10–15% of its original biological activity following a 14-day retention within a mini osmotic pump reservoir [77]. Stabilizing additives, such as antioxidants or acidifying solutions, can induce adverse effects on the host brain tissue [85]. A common problem for peptides is precipitation when maintained at concentrations necessary for infusion. At the end of the infusion period, it is recommended that the concentration and biologic activity of the molecule(s) in the infusate be verified. Pump failures from either the infusion module or an occluded cannula can lead to either a lack of drug delivery or a purge of the neuroactive factor with a potential for toxicity. In long-term experiments, pump-derived cytotoxins, in conjunction with elevated infusion rates, have been shown to contribute to ablated tissue at the site of the cannula [86]. As a point source delivery method to the tissue parenchyma, the diffusion distance as a function of the concentration gradient results in a sharp decrease in the concentration of the drug over a short distance. Lastly, for long-term experiments, pumps must be refilled, leading to an increase in the risk of infection.

2.6. Sustained-release polymer systems

Neuroactive molecules entrapped within various polymer systems have shown the ability to maintain a sustained release profile from weeks to months [87–90]. Sustained release of L-dopa or dopamine has been delivered into the brain of experimentally induced animal models of PD utilizing resorbable polyester copolymer spheres of lactide and glycolide [87], ethylene vinyl acetate copolymers (EVAc) [88,89], and silicone elastomers [91]. Each system provided a sustained release profile that resulted in a significant improvement in receptor-mediated rotational behavior in rats. The EVAc system has also been utilized for the sustained release of substance P [92] which provided neuroprotection to striatal neurons against quinolinic acid, an animal model of Huntingon's disease. By releasing GABA from EVAc rods, Kokaia et al. [93] also demonstrated the ability to suppress epileptic seizures in kindling rat models.

Trophic factors, specifically nerve growth factor (NGF), has been the target for many sustained release polymer systems. Hoffman et al. [41] exhibited sustained release of mouse NGF from EVAc rods for several weeks. Following implantation into a rudimentary rat model of AD, the NGF releasing rods also exhibited the ability to rescue a transected fimbria fornix, preventing the death of cholinergic neurons in the septum that are connected by the fimbria fornix to the hippocampus [41]. Sustained-release polymer systems have more recently been utilized in conjunction with NGF–dextran conjugates to not only regulate or manipulate the release rate of NGF delivery to the tissue, but moreover, the clearance rate of the NGF–dextran conjugate was significantly lower than the NGF alone. Hence, manipulation of the NGF or other trophic factors can improve penetration and retention in the brain [94]. A recent study has also shown the ability to maintain a sustained release of bioactive NGF from bioresorbable polyester microspheres for up to a 91-day duration [90].

Sustained-release polymer systems offer the ability to be implanted safely and are well tolerated by the host [88]. They can be retained as a closed system within a host system and thus do not provide a conduit to contamination or infection. However, the polymer systems exhibit a finite capacity and must be replaced or supplemented to achieve long-term release, an option that is undesirable. Sustained-release polymer systems have been an effective strategy for delivering chemotherapeutic agents in a site-specific manner for the treatment against brain cancer such as recurrent gliomas [95].

2.7. Cell therapy

The successful application of cell therapy, or cellular transplantation, to the damaged or deficient

CNS to restore form and function in preclinical injury models and clinical situations has been demonstrated in many instances [8,96]. Clinical transplants of adrenal medullary autografts and fetal nigral tissue in PD were based on the idea that the missing neurotransmitter dopamine by the cells in the neural grafts [97]. Cellular transplants that provide a local source of neurotransmitters and/or trophic factors (i.e. neurotrophins) by these so-called cellular 'minipumps' is one of the foundations of transplantation therapies in the CNS. Fetal nigral grafts have shown the ability to establish a functional reinnervation, thus replacing the damaged neuronal circuitry [8]. However, the dopamine neurons must be transplanted to the host striatum. To survive transplantation, fetal dopamine neurons must be isolated at an early stage of development, which is a technically difficult task. Neural grafts have also been utilized to provide axonal bridges for the repair of damaged spinal circuitry and glial elements to supplement the loss in sclerotic lesions. The focus of this chapter is cell therapy as biologic minipumps to supply missing neurotransmitters or provide local neurotrophins as a neuroprotective strategy. Although cellular minipumps are thought to provide local delivery of neuroactive molecules in the therapeutic range, one of the potential limitations is dosing, and as described for the pump technology in tissue parenchyma, having a point source of factor limits diffusion distance. Cellular transplantation of genetically engineered cells to secrete a factor of choice, with and without the use of polymer encapsulation technology, is presented in Section 3.

2.8. Direct gene therapy

Research for expanding our understanding of genetic and biochemical deficiencies of various CNS disorders has been extensive over the past decade and provided a rationale basis to the direct approach for gene therapy. The direct transfer of a therapeutic gene via viral vector-mediated methods, or the transplantation of genetically engineered cells, with and without an encapsulated membrane, and the implantation of fetal or engineered progenitor cells are some of the technologies being developed to produce and deliver a specific enzyme, neurotransmitter or neurotrophin for the dysfunctional CNS [3,48]. Although much closer to providing a clinical reality, issues such as regulation of the cellular expression of the transgene, potential for toxicity of the viral proteins, and host immunology must be addressed.

3. A model system: therapies for Parkinson's disease

3.1. Overview

Of the neurological disorders of the CNS, PD is probably best understood as is reflected in the availability of clinical treatments. PD affects approximately 500 000 individuals. The cardinal signs of PD are characterized as muscle rigidity, tremor and bradykinesia. PD progresses with time, affecting gait and posture, and may lead to dementia. PD is distinguished pathologically by the presence of Lewy bodies in the substantia nigra and nigral cell loss. Lewy bodies are spherical inclusions between 5 and 25 μm in diameter. PD results from a neurological deficiency of dopamine. It has been effectively treated with systemic L-dopa (precursor to dopamine) which, unlike dopamine, can cross the BBB. L-Dopa is converted to dopamine in the brain. L-Dopa is administered systemically in large doses because some is degraded in the periphery (by decarboxylation), prior to crossing the BBB [98]. Over time, L-dopa loses its efficacy and results in dose-related side effects. While not ideal for patients with advanced stage PD, at least some relief is available to patients suffering from PD. This is not true for other disorders. Some of the advances in PD have taken advantage of an accurate primate model that relies on the administration of the heroin analog, l-methyl-4-phenyl-l,2,3,6-tetrahydropyridine (MPTP) [99]. Having a reliable model allows therapeutic strategies, such as cell therapies, to be compared to traditional delivery methods. Table 2 summarizes the advantages and disadvantages of encapsulated and non-encapsulated cell therapies.

3.2. Cellular transplants

Neural transplantation requires that cells be implanted into damaged areas of the CNS. In initial

Table 2
Comparison of the advantages and disadvantages of transplanting of unencapsulated and encapsulated cells

Unencapsulated cell therapy	Encapsulated cell therapy
Advantages	
Anatomical integration between host and transplanted tissue	Use of allo- and xeno grafts without immunosuppression
Good cell viability and neurochemical diffusion	Good cell viability
	Thin wall and spherical shape are optimal for neurochemical diffusion;[a] mechanical stability is optimal for retrievability[b]
Disadvantages	
Requires immunosuppression	Capsule dimensions may limit neurochemical diffusion and cell viability
Tissue availability limited	Need for multiple implants may produce significant tissue
Limited retrievability	Limited retrievability[a]
Societal and ethical issues associated with fetal tissue	

[a] Applies to microencapsulated cells only.
[b] Applies to macroencapsulated cells only.

studies, fetal tissue was transplanted in animals to determine whether the tissue could survive, integrate with the host tissue and promote functional recovery. Early results indicated that all three goals could be achieved; the grafted neural tissue attenuated the functional deficits in animals having experimentally induced CNS lesions [100–102]. Notwithstanding the limited tissue availability and ethical issues associated with using human fetal tissue has slowed progress in this area, the results are encouraging and may lay the foundation for clinical treatment one day.

For PD, dopamine-producing cells are implanted in the striatum, thereby substituting for the lost nigrostriatal neurons. Embryonic rodent and non-human primate neurons integrated, respectively, in the rat and primate brain and restored dopaminergic function in the area surrounding the transplant; however, early development stage neurons were required for survival [8]. Perhaps even more exciting are the human clinical data that demonstrate survival and functioning of human embryonic mesencephalic dopaminergic neurons transplanted in adult brains (in the striatum) of individuals suffering from PD. Transplanted grafts survived for over 6 years while the patients own dopamine neurons continued to degenerate. Most of the patients treated in this way demonstrated improved PD symptoms, and some were able to withdraw L-dopa treatment; however, overall, the results indicate that this technique is neither efficacious nor reproducible for widespread use. To realize dopamine cell transplantation as a viable technique to treat PD, strategies must increase cell survival and innervation of the striatum, reconstruct the nigrostriatal pathway and use alternate cell sources [103].

3.3. Enhancing transplant viability

The limited survivability of transplanted neurons makes current strategies unfeasible. While $\sim 50\%$ of fetal CNS cells die naturally, $< 5\%$ of transplanted neurons survive. Thus tissue from at least three embryos is required for transplantation on each side of the brain for therapeutic efficacy. To overcome the low survival rate of dopaminergic neurons transplanted in the striatum, testis-derived Sertoli cells have been co-transplanted [104]. The Sertoli cells

provide trophic support to the neurons, thereby promoting their survival. Alternate strategies of trophic support are also being investigated. The following have been administered in rats and have enhanced the survival and growth of transplanted dopaminergic neurons: glial-cell-line-derived neurotrophic factor (GDNF), brain-derived neurotrophic factor (BDNF) and basic fibroblast growth factor (bFGF) [105]. By increasing survival by 2–3 fold, the amount of tissue required for transplantation can be reduced.

Alternate methods have been investigated to enhance survivability of transplanted cells. Caspases are proteases that are activated in one of the final stages before a neuron is committed to die by apoptosis. Pharmacological inhibitors of caspases are currently under investigation for prolonged survival of transplanted cells [106]. Additional sources of growth factors have also been investigated. For example, transgenic mice were developed to have astrocytic stem cells capable of producing human nerve growth factor (hNGF) [107]. These stem cells were transplanted in adult rats and hNGF was detected immunochemically within 1 month of transplantation. This technique, while nascent, may be appropriate for delivery of neurotrophins to the CNS.

3.4. Alternate transplant sources

Due to ethical and fetal tissue availability issues, alternative cell sources have been investigated. For example, the adrenal medulla has been used as a source of cells because they produce catecholamines and can transform into a neuronal phenotype. While adrenal grafts survive poorly in the striatum, they induce sprouting of host-derived fibers in the caudate nucleus [23]. To enhance survivability of the adrenal medulla grafts, peripheral nerve was cotransplanted in five patients. Specifically, adrenal medullary tissue and minced intercostal nerve was implanted in the striatum after which patients were followed for 2 years. Clinical improvements were observed with these autologous cografts [108]. A separate effort to improve adrenal medulla tissue survival transplanted adrenal chromaffin cells on microcarrier beads in rats. Functional recovery was observed for 1 year post-transplantation relative to controls [109]. Human CNS progenitor cells that were transplanted in adult rats differentiated into both neurons and astrocytes following intracerebral grafting, indicating them as a potential source of cells for neural transplantation [110].

While autogeneic tissue is preferable over xenogeneic tissue in terms of host immune response, autogeneic tissue is limited in supply, as is fetal allogeneic tissue. Transplantation of xenogeneic tissue presents several challenges (see Section 3.5), yet is abundant. Fetal pig dopaminergic neurons were implanted in the caudate-putamen brain region of a patient suffering from Parkinson's disease [111]. After 7 months, the porcine neurons survived and extended axons into the host brain tissue. Interestingly, only a low reactivity of microglia and T-cells were observed in direct proximity to the grafts. The immunoprivileged CNS allows cross-species transplants to survive with coadministration of immunosuppressive agents. However, because there are concerns relating to the efficacy of long-term immunosuppression of xenogeneic tissue and the transfer of infectious agents across species, xenogeneic transplants remain controversial [112].

An alternate source of tissue is cell lines that can be transfected to differentiate into neuron-like cells. For example, cells of the human embryonal carcinoma cell line, NT2N, were implanted into CNS tissue and shown to survive for more than 1 year in immunodeficient mice [113]. Although these are tumorigenic cells, they may be suitable for gene therapy against CNS diseases. Additional gene therapy strategies are under investigation using the mouse embryonal carcinoma cell line, P19 [114] and genetically modified fibroblasts [115].

3.5. Polymer-encapsulated cell therapy

Cells or cell clusters are contained within a semipermeable membrane that permits the diffusion of small molecule nutrients, such as insulin and oxygen, but limits that of larger molecules, such as immunoglobulin G (IgG) and M (1gM). At the same time, the bioactive agents produced by the cells are able to diffuse across the membrane and into the host. The membrane isolates the enclosed cells from the host immune system, providing the cells with 'stealth-like' properties. This allows a plethora of cell types to be transplanted without the use of

immunosuppressants. Encapsulated cell therapy has been studied most extensively for the treatment of diabetes, using insulin-secreting cells, and PD, using dopamine-secreting cells [116].

As has been described in Chapter 5, there are two methods of encapsulation — micro and macro — each with its own advantages and disadvantages. Cells that are microencapsulated have a thin, spherical semipermeable shell surrounding them [117,118]. This is advantageous for diffusion across the membrane and cell viability, but in some types of processing, disadvantageous because cells can be exposed to organic solvents. Other types of microcapsules, e.g. the polyelectrolytes, can be mechanically fragile and chemically unstable. Cells that are macroencapsulated have a selectively permeable, usually cylindrical, membrane surrounding them [119]. Cells within the hollow fiber membrane (HFM) are usually suspended in or supported by a matrix. The ends of the HFM are sealed, thereby forming a capsule around the cells within. This technique is advantageous for implant stability and retrievability, but disadvantageous because the thicker wall membrane increases diffusion distance across the membrane, thereby limiting cell viability and decreasing the release of bioactive molecules. The stability of both the membrane [120] and the matrix [121] used within the device to suspend encapsulated cells are essential to the optimal functioning of the device. Without stable implant materials, the cells would be exposed to the host immune system and suffer the same consequences of non-encapsulated cells. Fig. 1 summarizes the different geometries used in encapsulated cell therapy.

To overcome the depletion of dopamine in the striatum of Parkinsonian patients, dopamine-secreting cells are encapsulated in an immunoisolatory polymeric membrane and then implanted directly in the striatum. This allows the de novo synthesized dopamine, produced by the cells, to be delivered directly to the target site. The dopamine diffuses across the membrane and into the surrounding tissue. The major challenges of encapsulated cell therapy include: host tissue reaction to the encapsulated cells in the absence of immunosuppressants; continued viability and functioning of the encapsulated cells; effectiveness of cell therapy. Each of these will be discussed in more detail below.

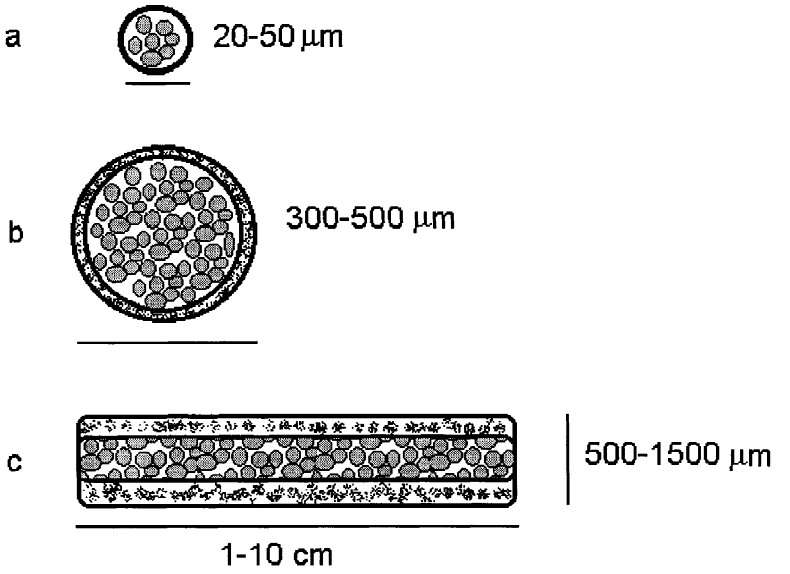

Fig. 1. Encapsulated cell therapy strategies immunoisolate cells from the host tissue within membranes that can adopt several shapes and dimensions. Most notably, these include: (a) conformal coatings that include tens of cells within a thin shell; (b) microcapsules that house several hundred cells within a sphere; (c) macrocapsules which house several thousands of cells within a hollow fiber membrane. While flatsheet membranes have also been investigated in other cell delivery strategies, HFMs have been used for cell delivery to the CNS. All strategies incorporate matrix materials to suspend cells evenly within capsule geometry.

3.6. Cell therapy matrices

Several cell types have been investigated in encapsulated therapy: (1) primary postmitotic cells, such as bovine adrenal chromaffin cells (BACs), for the treatment of chronic pain [30]; (2) immortalized (or dividing) cells, such as pheochromocytoma (PC 12) cells, for the treatment of PD [13,14,139]; and (3) engineered cell lines, such as baby hampster kidney (BHK) cells that have been engineered to produce human nerve growth factor (hNGF) for the treatment of AD [17]. While dividing cell lines are advantageous for cell sourcing and sterility-testing, post-mitotic cells do not overgrow the capsule milieu.

Strategies have been employed to control the growth of cells within the capsule. Just as the extracellular matrix (ECM) regulates cell function in vivo [122], matrix materials have been encapsulated within the membrane capsules to influence cell viability. For example, BACs have been immobilized in alginate to prevent aggregation which, in turn, reduces central necrotic cores from forming [123]. Similarly, PC12 cells have been distributed within precipitated chitosan which provides a scaffolding structure on which the cells anchor [124].

The matrix material can be manipulated to influence specific cellular functions, such as cell attachment, differentiation or proliferation. For example, peptides, such as arginine–glycine–aspartic acid (RGD) have been immobilized on a variety of surfaces to promote cell adhesion [125]. Integrin receptors on the cell surface membrane are known to interact with the RGD sequence that is found in fibronectin, among other proteins. Similarly, tyrosine–isoleucine–glycine–serine–arginine (YIGSR) and isoleucine–lysine–valine–alanine–valine (IKVAV), both of which are found in laminin, have been immobilized on surfaces [126] and gels [127] to promote neuronal cell adhesion and neurite outgrowth.

Each cell type requires a specific matrix material for optimal cell functioning once encapsulated. For primary cells, the matrix material can enhance the survival of one cell type over another, which is advantageous for the overall functionality of the device. For example, post-mitotic primary cells (e.g. BACs) thrive in alginate whereas mitotically-active fibroblasts do not. The use of alginate is essential to the optimal functioning of this device since some fibroblasts are always isolated with BACs. In the absence of alginate or other immobilizing matrices, the fibroblasts can expand and overgrow the encapsulated milieu, resulting in a device deficient in bioactive factors produced from the chromaffin cells [128]. Alternatively, BHK cells, a fibroblastic cell line, thrive in collagen, such as Vitrogen® 100, while PC12 cells thrive in precipitated chitosan [124].

3.7. Transplant longevity

PC12 cells have been investigated in both micro- [129] and macro- [130] encapsulated geometries for therapeutic efficacy in animal models of PD. PC12 cells, originally derived from a rat medullary tumor, secrete high levels of dopamine under both basal and, especially under chemical-evoked conditions. Encapsulated PC12 cells have been maintained both in vitro and in vivo for over 6 months during which time they continue to produce and release dopamine. When exposed to 56 mM potassium, encapsulated PCI2 cells retain the ability to significantly increase their output of dopamine.

Positron emission tomography (PET) was used to confirm that encapsulated PC12 cells produce L-dopa in situ in MPTP-treated non-human primates [131]. By imaging before and after implantation of encapsulated PC12 cells, it was clear that PC12 cells store, re-uptake and functionally replenish dopamine in the host tissue. PET thus serves as a useful and non-invasive tool to monitor device performance.

PC12 cell longevity has been proven in xenogeneic models, thereby underscoring the importance of the membrane structure for immunoisolation. For example, when encapsulated PC12 cells were implanted using intact devices into the striatum of guinea pigs, there was a minimal astrocytic response, as determined by GFAP immunolabeling, and no evidence of lymphocyte infiltration of the device. In contrast, when specifically damaged devices were used, there was limited cell survival, demonstrable inflammation and lymphocytes invading the device. Without immunosuppression, non-encapsulated PC12 cells did not survive implantation in either the guinea pig or non-human primate

striatum whereas encapsulated PC12 cells survived for 6 months in non-human primate brains [132].

3.8. Treatments for other CNS disorders

Both encapsulated and non-encapsulated strategies have been pursued for other CNS disorders. For example, fetal cerebellar grafts that were transplanted in Purkinje cell-deficient mice resulted in improved motor behavior, demonstrating the potential of this technique for patients with cerebellar degeneration. Using human fetal cerebellar tissue, an organotypic folia-like organization was observed in nude mice, demonstrating that these cells can organize into the cellular layers associated with the normal cerebellum [133]. Fetal spinal cord tissue, transplanted in rats, has been shown to rescue axotomized neurons and promote their regeneration [134]. In addition to providing a conduit for axonal bridging, spinal implants have provided a source of cellular replacements for lower motor neurons [135], myelin-producing cells [26,27,136], restoration of motor functions with monoaminergic cell implants [137,138] and grafts for pain modulation [28–31]. In addition to pain modulation, encapsulated cell therapies have been investigated for treatments of Alzheimer's disease and Huntington's disease.

Alzheimer's disease is the most prevalent form of adult onset of dementia, affecting $\sim 5\%$ of the adult population over 65 years. It results in the progressive deterioration of cognitive ability and memory, which is related, at least in part, to the degeneration of basal forebrain cholinergic neurons. Several studies have indicated that NGF delivery may be useful in the treatment of AD. While no model system captures the complex etiology of AD, model systems have been developed to determine whether delivery of NGF prevents cholinergic neuron death following acute trauma. For example, BHK cells transfected to produce abundant, stable levels of hNGF, were encapsulated in a hollow fiber membrane and implanted in the ventricle of rats following aspiration of the fimbria/fornix [17]. Compared to controls (encapsulated implants containing transfected BHK cells) in which 14% of the neurons remained viable on the lesioned side, BHK–NGF-encapsulated cells were capable of saving 88% of the cholinergic neurons. Similar results were observed in non-human primates, indicating the promise of this technique for clinical applications [139].

Huntington's disease (HD) is a progressive, inherited neurological disorder characterized by severe degeneration of the basal ganglia neurons and particularly those of the striatum. Severe, uncontrollable motor abnormalities, abnormal postures, and a progressive dementia are associated with HD and ultimately result in death within 15–17 years of the time of onset. Currently, no treatments adequately control the behavioral symptoms, nor effectively alter the neurodegenerative process. Since genetic screening is now available to identify those at risk for HD, there is a unique opportunity to design, characterize and implement therapeutic strategies to alter the time course for striatal degeneration.

An effective model of HD is achieved with intrastriatal injections of quinolinic acid (QA) which has been used to evaluate therapeutic strategies [140]. As a potential strategy for neuroprotection associated with NGF and CNTF, BHK–NGF and BHK–CNTF encapsulated cells were implanted in rat ventricles of healthy adult rats [22,141], and 1 week later, these animals received unilateral injections of QA (or saline in controls) in the ipsilateral striatum. Compared to controls, not only was the lesion significantly reduced in those animals having BHK–NGF or BHK–CNTF encapsulated cells, but the extent of host neural damage normally resulting from QA was also significantly reduced. Similar results were obtained with BHK–CNTF encapsulated cells implanted in non-human primates [24]; however, when capsules were placed directly in the brain parenchyma, striatal neurons were not protected, suggesting that diffusion is a key factor for efficacy and ultimate clinical application of this therapeutic strategy [23].

4. Host responses to cell therapies

4.1. Overview

Transplant survival in the CNS, with and without an encapsulating membrane, is mediated by many factors. The host's cellular and tissue response(s) to cell therapy applications profoundly impacts successful outcomes. For example, intracerebral neural

grafts of tissue/cells across a species barrier (i.e. xenografts) without an encapsulating membrane or immunosuppressive therapies are rejected by the host's immune system [13,14]. In addition, the cellular/tissue reaction mediated by the host in response to a foreign body determines the compatibility of the transplant, typically referred to as biocompatibility.

4.2. Biocompatibility and immune issues

Implantation of foreign material into the body elicits an inflammatory response, the extent of which is affected by the implant size, shape and composition and the host tissue in which it is implanted. The inflammatory response is often heightened to rough surfaces and edges vs. smooth ones. Residuals, such as monomers, solvents, processing aids, will elicit an inflammatory response if leached out of a polymeric structure in vivo. The CNS is considered to be a privileged transplant site immunologically. Privileged to the extent that factors unique to the CNS, such as the presence of the BBB, the low expression of major histocompatibility complex products on nervous tissue, and production of local immunosuppressive factors by CNS cells, modify the course of transplant survival more readily than in peripheral sites. However, even with the immunoprivileged status, in cases of transplants across species barriers, i.e. xenotransplants (xenografts), transplant survival is dependent on concurrent immunosuppressive drug therapy.

Encapsulation devices have been prepared where only a minor inflammatory response has been observed [88]. There was minimal necrotic tissue surrounding the polymeric capsule that was implanted in the striatum of rodents. The reactive astrocytes were labeled with glial fibrillary acidic protein (GFAP). Those astrocytes surrounding the implant at 2 weeks, diminished by 4 weeks to a minimal gliotic reaction. A minimal host tissue response is critical to optimal functioning of an implanted device for the continued transport across the membrane of both nutrients for cell viability and bioactive cell products for therapeutic efficacy.

Notwithstanding the minimal host tissue response observed, efforts to further enhance biocompatibility or reduce protein adsorption have been pursued. For example, poly(acrylonitrile–co-vinyl chloride) (PAN–VC) has been used extensively for the macroencapsulation of cells. When PAN–VC devices are maintained in serum-free medium prior to implantation, there is a minimal host tissue response; however, when similar devices are immersed in serum-rich medium prior to implantation, a host tissue reaction is evoked. By decreasing protein adsorption to PAN–VC devices, the handling of the implants may become less restrictive which, in turn, may extend their shelf-life. To this end, PAN–VC hollow fiber membranes were modified by grafting poly(ethylene oxide) (PEO) and shown both to adsorb less protein and to have slightly better biocompatibility with brain tissue than unmodified PAN–VC controls [142]. Similar studies have been conducted with poly(hydroxyethyl methacrylate–co-methyl methacrylate) (PHEMA–MMA), which has been used extensively for microencapsulation of cells. PHEMA–MMA was modified with PEO and shown to have decreased protein adsorption relative to controls [118].

4.3. Behavioral outcome

Animal behaviour has been assessed extensively in order to gain a better perspective on the efficacy of encapsulated PC12 cell therapy for PD. Both unilateral 6-hydroxy dopamine-lesioned rodents and MPTP-lesioned non-human primates have been evaluated. The data demonstrates that the catecholamines secreted from encapsulated PC12 cells have therapeutic potential at the behavioral level [132,143]. For example, when encapsulated PC12 cells were implanted in rodents with dopamine-depleted striata, they exhibited 40–50% fewer rotations after apomorphine administration than non-implanted control rats, thereby indicating that the catecholamines released from PC12 cells were sufficient to reduce the degree of synaptic supersensitivity that develops after dopamine-depleting lesions. The effectiveness of the devices on rodent behaviour was evident for up to 6 months and only as long as the devices remained in the striatum. Microdialysis confirmed dopamine production up to 200 μm from implanted macrocapsules and in concentrations similar to those obtained in control rats without lesioned striata [144]. A change in behaviour was observed

neither in rodents that received empty macrocapsule controls nor in rodents that had PC12 cell-loaded devices implanted in lateral ventricles [145].

While one can conclude that the L-dopa and dopamine produced by encapsulated PC12 cells leads to some behavioral recovery, these animal models have been criticized for a lack of clinical relevance and specificity. To this end, encapsulated PC12 cells were studied in terms of a series of non-drug induced behaviors. For example, since a transplantation procedure would be utilized with L-dopa administration, encapsulated PC12 cells were examined in terms of both behavioral measures and the therapeutic window of oral L-dopa administration (i.e. administered as Sinemet®. Encapsulated PC12 cells were implanted in the striatum of rats with unilateral dopamine depletions and evaluated in a number of behavioral assays over a range of oral Sinemet. The results indicated that the therapeutic effect was greater in rats that had encapsulated PC12 cell-implants than those that received oral Sinemet. Encouragingly, there was a beneficial, additive effect in rodents that received both treatments [146].

Additional studies have evaluated the efficacy of encapsulated PC12 cells in MPTP-lesioned non-human primates, which provide a more clinically relevant model due to size and complexity of their nervous system. Prior to lesioning, cynomolgus monkeys were trained to extend an arm and use their digits to pick up food from small wells. After lesioning, the monkeys were impaired in their ability to retrieve the food from the wells using their contralateral limb. Of four animals studied, three received PC12 cell implants and one received an empty control macrocapsule, the latter of which was ineffective in overcoming the deficit induced with MPTP-lesioning. Of the three PC12 cell implanted monkeys, two were able to perform the task at near normal levels for over 6 months post-transplantation. Interestingly, those animals (two out of three) that demonstrated improved motor control had capsules that, upon retrieval after 6.5 months, continued to secrete high levels of L-dopa and dopamine, with an abundance of viable PC12 cells distributed randomly throughout the capsule (Fig. 2). However, the animal that did not demonstrate improved motor control had few viable PC12 cells in retrieved capsules [132].

Thus, while the lack of behavioral efficacy in a non recovering monkey was explained by the lack of cell viability in those macrocapsules, this outcome highlights some of the problems associated with PC12 cells and their use as a source of cells for the delivery of therapeutic levels of L-dopa and dopamine for the treatment of PD. The variability in catecholamine production has plagued the use of PC12 cells while the inconsistency in viability of encapsulated cells has been problematic for this treatment overall. Several quality control measures have been developed to assess a device prior to implantation; however, these inherently increase the handling of devices, which complicates sterility and shelf-life.

Lastly, studies describing the behavioral outcome associated with an encapsulated cell therapy for AD in a rat model will be discussed. The aged rat exhibits basal forebrain cholinergic neuronal degeneration with cognitive impairments. A spatial learning task in a Morris water maze was utilized to train and assess cognitive functions in 3-, 18- and 24-month-old rats [18]. Cognitive functions declined with age and age-related atrophy of cholinergic neurons were most severe in animals exhibiting the greatest cognitive impairments. Following the training regime, the animals from the three age groups received bilateral ventricular implants of encapsulated BHK±NGF cells. No alterations were observed in the young non-impaired animals that received the NGF-secreting cellular implants. A significant improvement in cognitive functions was observed in the 18 and 24 month-old rats receiving the NGF implants associated with increases in the size of the atrophied cholinergic neurons. Furthermore, nanogram quantities of NGF were measured in the assay medium of retrieved NGF devices, providing evidence that the implants were functional for the 40-day duration. Moreover, the animals receiving BHK–NGF cells did not exhibit changes in mortality, body weights, activity levels, somatosensory thresholds, or hyperalgesia, further indicating that the NGF was not harmful to the rats.

In a related study [147], the long-term (i.e. longer than 1 year) behavioral consequences of sustained intraventricular release from BHK–NGF devices was evaluated for 13.5 months in healthy young adult rats. Following the 13.5-month implant interval, PCR analyses revealed that the NGF transgene copy number from the recovered NGF cells was equivalent to preimplant levels, indicating NGF gene

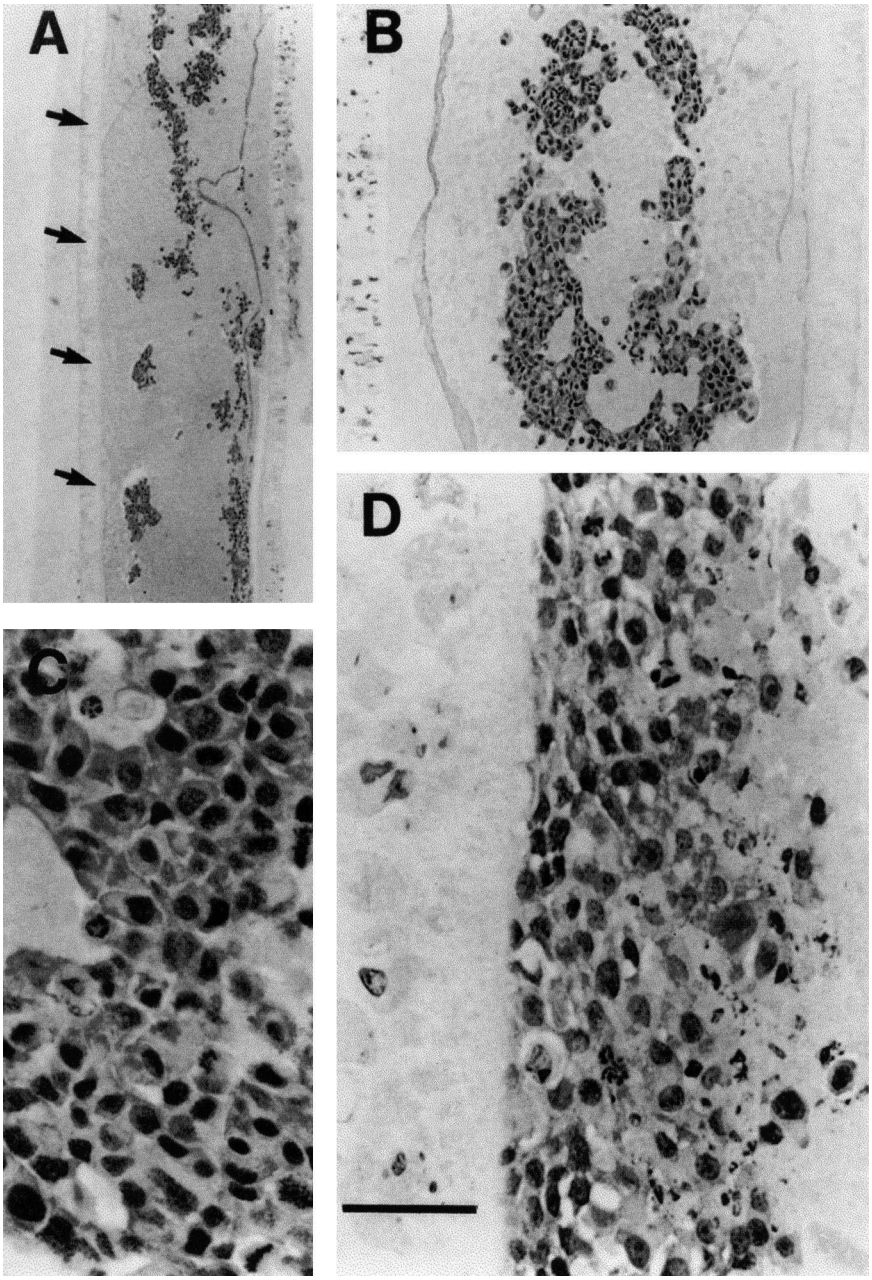

Fig. 2. Sections of (A) low, (B) medium and (C,D) high power photomicrographs of H + E-stained, methacrylate-embedded specimens demonstrating the presence of abundant viable PC12 cells within a polymer capsule 6.5 months following transplantation in the striatum of a nonhuman primate. Arrows (in A) delineate the wall of the polymer capsule. Scale bar in (D) = 50 μm. Reproduced from Ref. [132] with permission.

stability. As measured by ELISA, the NGF released from encapsulated cells into the tissue culture medium was 3.6±0.8 ng/device/24 h prior to implantation and 2.2±0.4 ng/device/24 h upon removal from the rat lateral ventricles after the 13.5 month interval in vivo. The sustained release of NGF into the ventricles did not impact body weight, mortality rate, motor/ambulatory function, cognitive function as assessed with the Morris water maze, produce hyperalgesia or cause a delay in matching to position in healthy adult rats. Morphologic analysis of retrieved capsules revealed abundant, viable BHK–NGF cells throughout the capsule (Fig. 3). Additionally, a marked hypertrophy of cholinergic neurons was observed within the striatum and robust sprouting of cholinergic fibers was observed within the frontal cortex and lateral septum proximal to the implant (Fig. 4). These results supported the notion that encapsulated xenogeneic cells could provide a safe and sustained method for the long-term delivery of neurotrophic factors from encapsulated, engineered cells.

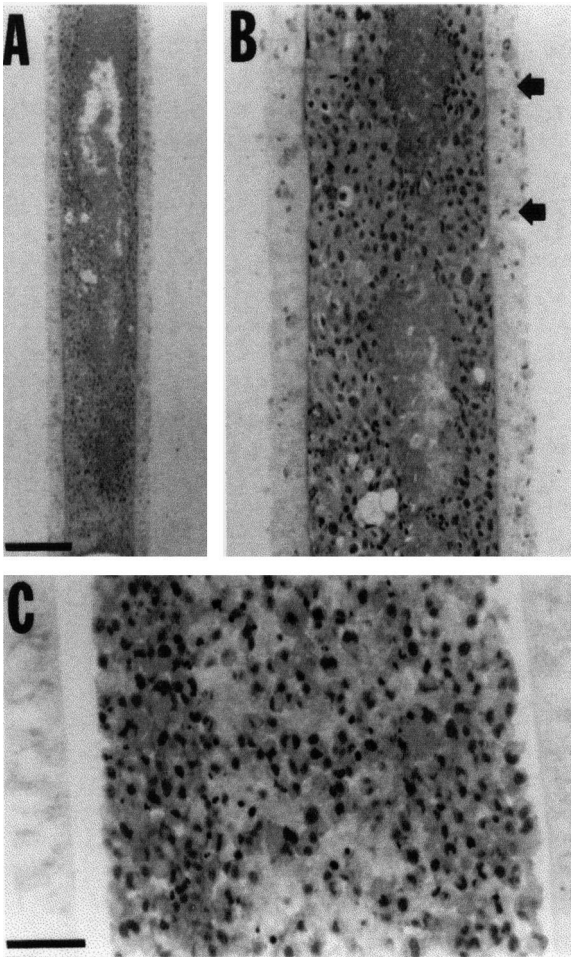

Fig. 3. Representative photomicrographs of BHK–NGF cells within a retrieved polymer capsule 13.5 months following implantation into a rat lateral ventricle. (A) Low, (B) medium and (C) high power photomicrographs of H & E-stained, methacrylate-embedded specimens demonstrating the presence of abundant viable BHK–NGF cells. Arrows (in B) illustrate the wall of the polymer capsule. Scale bars, A = 500 μm; C = 100 μm. Reproduced from Ref. [147] with permission.

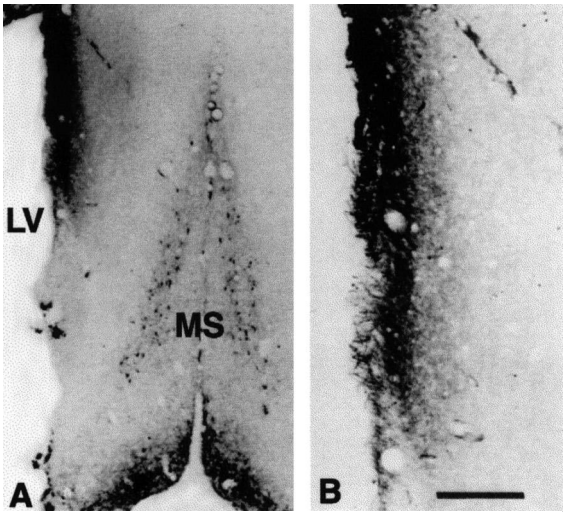

Fig. 4. Sprouting of cholinergic fibers in animals receiving polymer encapsulated BHK–NGF implants demonstrates the safety and efficacy of encapsulated xenogeneic cell therapy. (A) Low power photomicrograph of NGF receptor immunostained sections illustrating a dense plexus of cholinergic fibers on the side of NGF treatment along the dorsoventral extent of the septum. This plexus was most extensive in the dorsal quadrant. (B) High power photomicrograph illustrating the morphology of the NGF receptor immunoreactive fibers which coalesce as a dense bundle adjacent to the ventricular wall and the implant site. LV, lateral ventricle; MS, medial septum. Scale bar in B = 500 μm. Reproduced from Ref. [147] with permission.

5. Concluding remarks

Cell therapy with transplants of fetal neural tissue has been shown to be a useful strategy for the

treatment of a select group of human neurodegenerative disorders. However, the clinical utility of a fetal tissue strategy is confounded by societal and ethical issues, and additionally, by the ability to obtain sufficient quantities of quality controlled donor tissue. The site-specific application of neurotrophic factors to decelerate neural degeneration or potentially promote the regeneration of damaged CNS systems is an attractive avenue of research. Cell therapy utilized in conjunction with gene therapy provides a practical and quality assured alternative to the use of fetal tissue. An encapsulated cell therapy strategy offers the additional advantage of being retrievable to allow repeated and minimally invasive removal and replacement of devices over time. Regardless of whether traditional cell replacement or encapsulated strategies are followed, issues such as immunological compatibility, cell manipulation/expansion, safety and quality control [148], must be considered. When considering any novel therapy for treating CNS disorders, rigorous testing should be performed, to insure the therapy demonstrates clinical efficacy and safety. While each of therapeutic strategies described has its limitations and challenges, active research is leading to new advances which hold great promise for the future.

References

[1] D. Emerich, R. Michael, M. Lehman, P. Sanberg, Behavioral effects of neural transplantation, Cell Transplant 1 (1992) 401–427.

[2] R.J. Rubin, W.A. Gold, The Cost of Disorders of the Brain, National Foundation for Brain Research, 1992.

[3] Nature, 399 (Suppl.) A1–A47.

[4] S.D. Skaper, F.S. Walsh, Neurotrophic molecules: strategies for designing effective therapeutic molecules in neurodegeneration, Mol. Cell. Neurosci. 12 (1998) 179–193.

[5] J. Cervos-Navarro, S. Kannuki, Y. Nakagawa, Blood–brain barrier (BBB). Review from morphological aspect, Histol. Histopathol. 3 (2) (1988) 203–213.

[6] J.G. McComb, B.V. Zlokovic, Cerebrospinal fluid and the blood–brain interface, in: W.R. Cheek, A.E. Marlin, D.G. McLone, D.H. Reigel, M.L. Walker (Eds.), Pediatric Neurosurgery, W.B. Saunders, Philadelphia, (1994), pp. 167–185.

[7] L.L. Rubin, J.M. Staddon, The cell biology of the blood–brain barrier, Ann. Rev. Neurosci. 22 (1999) 11–28.

[8] S.B. Dunnett, A. Bjorklund, Functional Neural Transplantation, Raven, New York, (1994).

[9] A. Bjorklund, R.H. Schmidt, U. Stenevi, Functional reinnervation of the neostriatum in the adult rat by use of intraparenchymal grafting of dissociated cell suspensions from the substantia nigra, Cell Tissue Res. 211 (1980) 39–45.

[10] O. Lindvall, A. Bjorklund, Transplantation strategies in the treatment of Parkinson's disease: experimental basis and clinical trials, Acta Neurol. Scand. Suppl. 126 (1989) 197–210.

[11] W.J. Freed, M. Poltorak, J.B. Becker, Intracerebral adrenal medulla grafts: a review, Exp. Neurol. 110 (1990) 139–166.

[12] D.M. Yurek, J.R. Sladek, Dopamine cell replacement: Parkinson's disease, Annu. Rev. Neurosci. 13 (1990) 415–440.

[13] D.F. Emerich, S.R. Winn, L. Christenson, M.A. Palmatier, F.T. Gentile, P.R. Sanberg, A novel approach to neural transplantation in Parkinson's disease: use of polymer-encapsulated cell therapy, Neurosci. Biobehav. Rev. 16 (1992) 437–447.

[14] D. Emerich, R. Michael, M. Lehman, P. Sanberg, Behavioral effects of neural transplantation, Cell Transplant 1 (1992) 401–427.

[15] J. Springer, T. Collier, J. Sladek Jr., R. Loy, Transplantation of male mouse submaxillary gland increases survival of axotomized basal forebrain neurons, J. Neurosci. Res. 19 (1988) 291–296.

[16] I. Stromberg, C. Wetmore, T. Ebendal, P. Ernfors, H. Persson, L. Olson, Rescue of basal forebrain cholinergic neurons after implantation of genetically modified cells producing recombinant NGF, J. Neurosci. Res. 25 (1990) 405–411.

[17] S.R. Winn, J.P. Hammang, D.F. Emerich, A. Lee, R.D. Palmiter, E.E. Baetge, Polymer-encapsulated cells genetically modified to secrete human nerve growth factor promote the survival of axotomized septal cholinergic neurons, Proc. Natl. Acad. Sci. USA 91 (1994) 2324–2328.

[18] M.D. Lindner, C.E. Kearns, S.R. Winn, B. Frydel, D.F. Emerich, Effects of intraventricular encapsulated hNGF-secreting fibroblasts in aged rats, Cell Transpl. 5 (2) (1996) 205–223.

[19] J.H. Kordower, S.R. Winn, Y.T. Liu, E.J. Mufson, J.R. Sladek Jr., J.P. Hammang, E.E. Baetge, D.F. Emerich, The aged monkey basal forebrain: Rescue and sprouting of axotomized basal forebrain neurons after grafts of encapsulated cells secreting human nerve growth, Proc. Natl. Acad. Sci. USA 91 (1994) 10898–10902.

[20] J.M. Schumacher, M.P. Short, B.T. Hyman, X.O. Breakefield, O. Isacson, Intracerebral implantation of nerve growth factor-producing fibroblasts protects striatum against neurotoxic levels of excitatory amino acids, Neuroscience 45 (1991) 561–570.

[21] D. Frim, M. Short, W. Rosenberg, J. Simpson, X. Breakefield, O. Isacson, Local protective effects of nerve growth factor-secreting fibroblasts against excitotoxic lesions in the rat striatum, J. Neurosurg. 78 (1993) 267.

[22] D.F. Emerich, J.P. Hammang, E.E. Baetge, S.R. Winn, Implantation of polymer-encapsulated human nerve growth factor-secreting fibroblasts attenuates the behavioral and neuropathological consequences of quinolinic acid injections into rodent striatum, Exp. Neurol. 130 (1994a) 141–150.

[23] J.H. Kordower, C.G. Goetz, T.B. Freeman, C.W. Olanow, Dopaminergic transplants in patients with Parkinson's disease: neuroanatomical correlates of clinical recovery, Exp. Neurol. 144 (1997) 41–46.

[24] D.F. Emerich, S.R. Winn, P.M. Hantraye, M. Peschanski, E.-Y. Chen, Y. Chu, P. McDermott, E.E. Baetge, J.H. Kordower, Protective effects of encapsulated cells producing neurotrophic factor CNTF in a monkey model of Huntington's disease, Nature 386 (1997a) 395–399.

[25] P. Aebischer, M. Schleup, N. Deglon, J. Joseph, L. Hirt, B. Heyd, M. Goddard, J. Hammang, A. Zurn, A. Kato, F. Regli, E. Baetge, Intrathecal delivery of CNTF using encapsulated genetically modified xenogeneic cells in amyotrophic lateral sclerosis patients, Nat. Med. 2 (1996) 696–699.

[26] U. Tontsch, D. Archer, M. Dubois-Dalcq, I. Duncan, Transplantation of an oligodendrocyte cell line leading to extensive myelination, Proc. Natl. Acad. Sci. USA 91 (1994) 11616–11620.

[27] I. Duncan, Glial cell transplantation and remyelination of the central nervous system, Neuropathol. Appl. Neurobiol. 22 (1996) 87–100.

[28] J. Sagen, H. Wang, G.D. Pappas, Adrenal medullary tissue implants in rat spinal cord reduce nociception in a chronic pain model, Pain 42 (1990) 69–79.

[29] A.T. Hama, J. Sagen, Reduced pain-related behavior by adrenal medullary transplants in rats with experimental pain neuropathy, Pain 52 (1993) 223–231.

[30] P. Aebischer, E. Buschser, J. Joseph, J. Favre, N. deTribolet, M. Lysaght, S. Rudnick, M. Goddard, Transplantation in humans of encapsulated xenogeneic cells without immunosuppression — a preliminary report, Transplantation 58 (1994) 1275–1277.

[31] H. Wang, J. Sagen, Attenuation of pain-related hyperventilation in adjuvant arthritis rats with adrenal medullary transplants in the spinal sub-arachnoid space, Pain 63 (1995) 313–320.

[32] P.M. Friden, L.R. Walus, P. Watson, S.R. Doctrow, J.W. Kozarich, C. Backman, H. Bergman, B. Hoffer, F. Bloom, A.C. Granholm, Blood–brain barrier penetration and in vivo activity of an NGF conjugate, Science 259 (1993) 373–377.

[33] P.M. Friden, Receptor-mediated transport of therapeutics across the blood–brain barrier, Neurosurgery 35 (1994) 294–298.

[34] S. Jiao, P.J. Miller, P.A. Lapchak, Enhanced delivery of [^{125}I] glial cell line- derived neurotrophic factor to the rat CNS following osmotic blood–brain barrier modification, Neurosci. Lett. 220 (1996) 187–190.

[35] R.A. Kroll, E.A. Neuwelt, Outwitting the blood–brain barrier for therapeutic purposes: osmotic opening and other means, Neurosurgery 42 (1998) 1083–1099.

[36] J.H. Kordower, E.J. Mufson, A.C. Granholm, B. Hoffer, P. Friden, Delivery of trophic factors to the primate brain, Exp. Neurol. 124 (1993) 21–30.

[37] D.R. Riddle, L.C. Katz, D.C. Lo, Focal delivery of neurotrophins into the central nervous system using fluorescent latex microspheres, Biotechniques 23 (5) (1997) 928–937.

[38] E.J. Mufson, J.S. Kroin, T.J. Sendera, T. Sobreviela, Distribution and retrograde transport of trophic factors in the central nervous system: functional implications for the treatment of neurodegenerative diseases, Prog. Neurobiol. 57 (4) (1999) 451–484.

[39] H.L. Vahlsing, S. Varon, T. Hagg, B. Fass-Holmes, A. Dekker, M. Mamley, M. Mansthorpe, An improved device for continuous intraventricular infusions prevents the introduction of pump-derived toxins and increases the effectiveness of NGF treatments, Exp. Neurol. 105 (1989) 233–243.

[40] L. Olson, E.O. Backlund, T. Ebendal, R. Freedman, B. Hamberger, P. Hansson, B. Hoffer, U. Lindblom, B. Meyerson, I. Stromberg, Intraputaminal infusion of nerve growth factor to support adrenal medullary autografts in Parkinson's disease. One-year follow-up of first clinical trial, Arch. Neurol. 48 (1991) 373–381.

[41] D. Hoffman, L. Wahlberg, P. Aebischer, NGF released from a polymer matrix prevents loss of ChAT expression in basal forebrain neurons following a fimbria- fornix lesion, Exp. Neurol. 110 (1) (1990) 39–44.

[42] R. Langer, M. Moses, Biocompatible controlled release polymers for delivery of polypeptides and growth factors, J. Cell. Biochem. 45 (4) (1991) 340–345.

[43] M. Lindner, S. Winn, E. Baetge, J. Hammang, F. Gentile, E. Doherty, P. McDermott, B. Frydel, M. Ullman, T. Schallert, D. Emerich, Implantation of encapsulated catecholamine and GDNF-producing cells in rats with unilateral dopamine depletions and parkinsonian symptoms, Exp. Neurol. 132 (1995) 62–76.

[44] G. Sinson, M. Voddi, T. McIntosh, Combined fetal neural transplantation and nerve growth factor infusion: effects on neurological outcome following fluid-percussion brain injury in the rat, J. Neurosurg. 84 (4) (1996) 655–662.

[45] A. Martinez-Serrano, A. Bjorklund, Protection of the neostriatum against excitotoxic damage by neurotrophin-producing, genetically modified neural stem cells, J. Neurosci. 16 (15) (1996) 4604–4616.

[46] M. Tusznski, N. Weidner, M. McCormack, I. Miller, H. Powell, J. Conner, Grafts of genetically modified Schwann cells to the spinal cord: survival, axon growth, and myelination, Cell Transpl. 7 (2) (1998) 187–196.

[47] D. McTigue, P. Homer, B. Stokes, F. Gage, Neurotrophin-3 and brain-derived neurotrophic factor induce oligodendrocyte proliferation and myelination of regenerating axons in the contused adult rat spinal cord, J. Neurosci. 18 (14) (1998) 5354–5365.

[48] B.V. Zlokovic, M.L.J. Apuzzo, Cellular and molecular neurosurgery: Part II: vector systems and delivery methodologies for gene therapy of the central nervous system, Neurosurgery 40 (1997) 805–812.

[49] W. Bowers, D. Howard, H. Federoff, Gene therapeutic strategies for neuroprotection: implications for Parkinson's disease, Exp. Neurol. 144 (1) (1997) 58–68.

[50] M. Kaplitt, B. Darakchiev, M. During, Prospects for gene therapy in pediatric neurosurgery, Ped. Neurosurg. 28 (1) (1998) 3–14.

[51] M. Barkats, A. Bilang-Bleuel, M.H. Buc-Caron, M.N. Castel-Barthe, O. Corti, F. Finiels, P. Horellou, F. Revah, O.

Sabate, J. Mallet, Adenovirus in the brain: recent advances of gene therapy for neurodegenerative diseases, Prog. Neurobiol. 55 (4) (1998) 333–341.
[52] W. Banks, A. Kastin, Peptide transport systems for opiates across the blood– brain barrier, Neurosurgery 259 (1990) E1–E10.
[53] W. Pardridge, in: Peptide Drug Delivery To the Brain, Raven Press, New York, (1991), pp. 149–189.
[54] R. Broadwell, Endothelial cell biology and the enigma of transcytosis through the blood– brain barrier, Adv. Exp. Med. Biol. 331 (1993) 137–141.
[55] R. Broadwell, B. Balm, M. Salcman, Transcytotic pathway for blood-borne protein through the blood–brain barrier, Proc. Natl. Acad. Sci. USA 85 (1988) 632–636.
[56] W. Jefferies, M. Brandon, S. Hunt, S. Williams, K. Gatter, D. Mason, Transferrin receptor on endothelium of brain capillaries, Nature 312 (1984) 162–163.
[57] J. Fishman, J. Rubin, J. Handrahan, J. Connor, R. Fine, Receptor-mediated trancytosis of transferrin across the blood–brain barrier, J. Neurosci. Res. 18 (1987) 299–304.
[58] P. Friden, L. Walus, G. Musso, M. Taylor, B. Malfroy, R. Starzyk, Antitransferrin receptor antibody and antibody–drug, conjugates, cross the blood–brain barrier, Proc. Natl. Acad. Sci. USA 88 (1991) 4771–4775.
[59] J. Kordower, V. Charles, R. Bayer, R. Bartus, S. Putney, L. Walus, P. Friden, Intravenous administration of a transferrin receptor antibody–nerve growth factor conjugate prevents the degeneration of cholinergic striatal neurons in a model of Huntington's disease, Proc. Natl. Acad. Sci. USA 91 (1994) 9077–9080.
[60] S. Rapoport, Blood–Brain Barrier in Physiology and Medicine, Raven Press, New York, 1976.
[61] R. Blasberg, D. Groothuis, P. Molnar, A review of hyperosmotic blood– brain barrier disruption in seven experimental brain tumor models, in: B.B. Johansson, C. Owman, H. Widner (Eds.), Pathophysiology of the Blood–Brain Barrier, Elsevier, Amsterdam, (1990), pp. 197–220.
[62] S. Dahlborg, W. Henner, J. Crossen, M. Tableman, A. Petrillo, R. Braziel, E. Neuwelt, Non-ADS primary CNS lymphoma: the first examples of durable response in a primary brain tumor using enhanced chemotherapy delivery without cognitive loss and without radiotherapy, Cancer J. Sci. Am. 2 (1996) 166–174.
[63] P. Lapchak, D. Araujo, S. Carwell, F. Hefti, Distribution of [^{125}I] nerve growth factor in the rat brain following a single intraventricular injection correlation with the topographical distribution of trkA messenger RNA-expressing cells, Neuroscience 54 (1993) 445–460.
[64] P. Lapchak, K. Beck, D. Araujo, I. Irwin, J. Langston, F. Hefti, Chronic intranigral administration of brain-derived neurotrophic factors produces striatal dopaminergic hypofunction in unlesioned adult rats and fails to attenuate the decline of striatal dopaminergic function following medical forebrain bundle transection, Neuroscience 53 (1993) 639–650.
[65] Q. Yan, C. Matheson, J. Sun, M. Radeke, S. Feinstein, J. Miller, Distribution of intracerebral ventricularly administered neurotrophins in rat brain and its correlation with trk receptor expression, Exp. Neurol. 127 (1994) 23–36.
[66] K. Anderson, R. Alderson, C. Altar, P. DiStefano, T. Corcoran, R. Lindsay, S. Wiegand, Differential distribution of exogenous BDNF, NGF, and NT-3 in the brain corresponds to the relative abundance and distribution of high-affinity and low- affinity neurotrophin receptors, J. Comp. Neurol. 357 (1995) 296–317.
[67] L. Olson, A. Bjorklund, B. Hoffer, M. Palmer, A. Seiger, Spinal cord grafts: an intraocular approach to enigmas of nerve growth regulation, Brain Res. Bulletin 9 (1–6) (1982) 519–537.
[68] A.C. Granholm, Hippocampal transplants in oculo: a model for establishment of isolated circuits, in: P.M. Conn (Ed.), Methods in Neuroscience, Vol. 7, Academic, San Diego, 1991, pp. 327–345.
[69] A. Granholm, G. Gerhardt, M. Bygdeman, I. Stromberg, Human fetal xenografts of brainstem tissue containing locus coeruleus neurons: functional and structural studies of intraocular grafts in athymic nude rats, Exp. Neurol. 118 (1) (1992) 7–17.
[70] M. Eriksdotter-Nilsson, S. Skirboll, T. Ebendal, L. Olson, Nerve growth factor can influence growth of cortex cerebri and hippocampus: evidence from intraocular grafts, Neuroscience 30 (1989) 755–766.
[71] C. Backman, O. Biddle, T. Ebendal, P. Friden, G. Gerhardt, M. Henry, L. Mackerlova, S. Soderstrom, I. Stromberg, L. Walus, B. Hoffer, A. Granholm, Effects of transferrin receptor antibody–NGF conjugate on young and aged septal transplants in oculo, Exp. Neurol. 132 (1995) 1–15.
[72] H. Brem, R. Tamargo, A. Olivi, M. Pinn, J. Weingart, M. Wharam, J. Epstein, Biodegradable polymers for controlled delivery of chemotherapy with and without radiation therapy in the monkey brain, J. Neurosurg. 80 (2) (1994) 283–290.
[73] A. Ommaya, Implantable devices for chronic access and drug delivery to the central nervous system, Cancer Drug Deliv. 1 (2) (1984) 169–179.
[74] S. Stahl, Applications of new drug delivery technologies to Parkinson's disease and dopaminergic agents, J. Neural Trans. Suppl. 27 (1988) 123–132.
[75] M. Schwab, Experimental aspects of spinal cord regeneration, Current Opin. Neurol. Neurosurg. 6 (4) (1993) 549–553.
[76] T. Hagg, H. Vahlsing, M. Manthrope, S. Varon, Nerve growth factor infusion into the denervated adult rat hippocampal formation promotes its cholinergic reinnervation, J. Neurosci. 10 (1990) 3087–3092.
[77] X. Lu, T. Hagg, Glial cell line-derived neurotrophic facto prevents death, but not reductions in tyrosine hydroxylase of injured nigrostriatal neurons in adult rats, J. Comp. Neurol. 388 (1997) 484–494.
[78] A. Kearns, D. Gash, GDNF protects nigral dopamine neurons against 6-hydroxydopamine in vivo, Brain Res. 672 (1995) 104–111.
[79] T. Hagg, S. Varon, Ciliary neurotrophic factor prevents degeneration of adult rat substantia nigra dopaminergic neurons in vivo, Proc. Natl. Acad. Sci. USA 90 (1993) 6315–6319.

[80] T. Hagg, Neurotrophins prevent death and differentially affect tyrosine hydroxylase of adult rat nigrostriatal neurons in vivo, Exp. Neurol. 149 (1) (1998) 183–192.
[81] L. Williams, G. Inouye, V. Cummins, M. Pelleymounter, Glial cell line derived neurotrophic factor sustains axotomized basal forebrain cholinergic neurons in vivo: dose–response comparison to nerve growth factor and brain-derived neurotrophic factor, J. Pharmacol. Exp. Ther. 277 (1996) 1140–1151.
[82] M. Oudega, T. Hagg, Neurotrophins promote regeneration of sensory axons in the adult rat spinal cord, Brain Res. 818 (1999) 431–438.
[83] P. Dahm, P. Nitescu, L. Appelgren, I. Curelaru, Efficacy and technical complications of long-term continuous intraspinal infusions of opioid and/or bupivacine in refractory non-malignant pain: a comparison between the epidural and the intrathecal approach with externalized or implanted catheters and infusion pumps, Clin. J. Pain 14 (1998) 4–16.
[84] I. Angel, H. Gould Jr., M. Carey, Intrathecal morphine pump as a treatment option in chronic pain of nonmalignant origin, Surg. Neurol. 49 (1998) 92–98.
[85] J.G. DeYebens, S. Fahn, M.A. Mena, B. Pardo, M.J. Casarejos, Intracerebroventricular infusion of dopamine and its agonists in rodents and primates: an experimental approach to the treatment of Parkinson's disease, Trans. Am. Soc. Artif. Intern. Organs 34 (1988) 951–957.
[86] M. Bear, A. Kleinschmidt, Q. Gu, W. Singer, Disruption of experience-dependent synaptic modifications in striate cortex by infusion of an NMDA receptor antagonist, J. Neurosci. 10 (3) (1990) 909–925.
[87] A. McRae-Degueurce, S. Hjorth, D. Dillon, D. Maso, T. Tice, Implantable microencapsulated dopamine (DA): a new approach for slow-release DA delivery into brain tissue, Neurosci. Lett. 92 (3) (1988) 303–309.
[88] S.R. Winn, P. Aebischer, P.M. Galletti, Brain tissue reaction to permselective polymer capsules, J. Biomed. Mater. Res. 23 (1989) 31–44.
[89] A. Freese, B. Sabel, W. Saltzman, M. During, R. Langer, Controlled release of dopamine from polymeric brain implant: in vitro characterization, Exp. Neurol. 103 (1989) 234–238.
[90] X. Cao, M. Shoichet, Delivering neuroactive molecules from biodegradable microspheres for application in central nervous system disorders, Biomaterials 20 (1999) 329–339.
[91] J. Becker, T. Robinson, P. Barton, A. Sintov, R. Siden, R. Levy, Sustained behavioral recovery from unilateral nigrostriatal damage produced by the controlled release of dopamine from a silicone polymer pellet placed into the denervated striatum, Brain Res. 508 (1990) 60–64.
[92] P. Sanberg, D. Emerich, P. Aebischer, S. Amisetti, W. Ouellette, T. Koutouzis, D. Cahill, A. Norman, Substance P containing polymer implants protect against striatal excitotoxicity, Brain Res. 628 (1993) 327–329.
[93] M. Kokaia, P. Aebischer, E. Elmer, J. Bengzon, P. Kalen, Z. Kokaia, O. Lindvall, Seizure suppression in kindling epilepsy by intracerebral implants of GABA- but not by noradrenaline-releasing polymer matrices, Exp. Brain Res. 100 (1994) 385–394.
[94] C. Krewson, R. Dause, M. Michele, W. Saltzman, Stabilization of nerve growth factor in controlled release polymers and in tissue, J. Biomater. Sci. Polym. Ed. 8 (1996) 103–117.
[95] H. Brem, M. Mahaley Jr., N. Vick, K. Black, S. Schold Jr., P. Burger, A. Friedman, I. Ciric, T. Eller, J. Cozzens et al., Interstitial chemotherapy with drug polymer implants for the treatment of recurrent gliomas, J. Neurosurg. 74 (3) (1991) 441–446.
[96] S. Dunnett, Is it possible to repair the damaged prefrontal cortex by neural tissue transplantation?, Prog. Brain Res. 85 (1990) 285–297.
[97] S. Dunnett, A. Bjorklund, Prospects for new restorative and neuroprotective treatments in Parkinson's disease, Nature 399 (6738 Suppl) (1999) A32–39.
[98] O. Lindvall, P. Brundin, H. Widner, S. Rehncrona, B. Gustavii, R. Frackowiak et al., Grafts of fetal dopamine neurons survive and improve motor function in Parkinson's disease, Science 247 (1990) 574–577.
[99] J.W. Langston, Epidemiology versus genetics in Parkinson's disease: progress in resolving an age old debate, Ann. Neurol. 44 (suppl.) (1998) S45–S52.
[100] M.J. Perlow, W.J. Freed W., A. Seiger, L. Olson, R.J. Wyatt Wyat, Brain grafts reduce motor abnormalities produced by destruction of nigrostriatal dopamine system, Science 204 (1979) 643–647.
[101] A. Bjorklund, S.B. Dunnett, U. Stenevi, M.E. Lewis, S.D. Iversen, Reinnervation of the denervated striatum by substantia nigra transplants: functional consequences as revealed by pharmacological and sensorimtor testing, Brain Res. 199 (1980) 307–333.
[102] R.A. Bakay, D.L. Barrow, M.S. Fiandaca, P.M. luvone, A. Schiff, D.C. Collins, Biochemical and behavioral correction of MPTP Parkinson-like syndrome by fetal cell transplantation, Ann. NY Acad. Sci. 495 (1987) 623–640.
[103] O. Lindvall, Neural transplantation: a hope for patients with Parkinson's disease, Neuroreport 8 (1997) iii–x.
[104] A.E. Willing, A.I. Othberg, S. Saporta, A. Anton, S. Sinibaldi, S.G. Poulos, D.E. Cameron, T.B. Freeman, P.R. Sanberg, Sertoli cells enhance the survival of cotransplanted dopamine neurons, Brain Res. 822 (1999) 246–250.
[105] C. Rosenblad, A. Martinez-Serrano, A. Bjorklund, Glia cell line-derived neurotrophic factor increases survival, growth and function of intrastriatal fetal nigral dopaminergic grafts, Neuroscience 75 (1996) 979–985.
[106] Z. Boonman, O. Isacson, Apoptosis in neuronal development and transplantation: role of caspases and trophic factors, Exp. Neurol. 156 (1999) 1–15.
[107] M.K. Carpenter, C. Winkler, R. Fricker, D.F. Emerich, S.C. Wong, C. Greco, E.Y. Chen, Y. Chu, J.H. Kordower, A. Messing, A. Bjorklund, J.P. Hammang, Generation and transplantation of EGF-responsive neural stem cells derived from GFAPhNGF transgenic mice, Exp. Neurol. 148 (1997) 187–204.
[108] R.L. Watts, T. Subramanian, A. Freeman, C.G. Goetz, R.D. Penn, G.T. Stebbins, J.H. Kordower, R.A. Bakay, Effect of stereotaxic intrastriatal cografts of autologous adrenal

medulla and peripheral nerve in Parkinson's disease: two-year follow-up study, Exp. Neurol. 147 (1997) 510–517.
[109] C.V. Borlongan, S. Saporta, P.R. Sanberg, Intrastriatal transplantation of rat adrenal chromaffin cells seeded on microcarrier beads promote long-term functional recovery in hemiparkinsonian rats (published erratum appears in Exp. Neurol. (1998) 154, 259), Exp. Neurol. 151 (1998) 203–214.
[110] C.V. Svendsen, M.A. Caldwell, J. Shen, M.G. ter Borg, A.E. Rosser, P. Tyers, S. Karmiol, S.B. Dunnett, Long-term survival of human central nervous system progenitor cells transplanted into a rat model of Parkinson's disease, Exp. Neurol. 148 (1997) 135–146.
[111] T. Deacon, J. Schumacher, J. Dinsmore, C. Thomas, P. Palmer, S. Kott, A. Edge, D. Penney, S. Kassissieh, P. Dempsey, O. Isaacson, Histological evidence of fetal pig neural cell survival after transplantation into a patient with Parkinson's disease, Nat. Med. 3 (1997) 350–353.
[112] D. Butler, Last chance to stop and think on risks of xenotransplants, Nature 391 (1998) 320–324.
[113] J.Q. Trojanowski, S.R. Kleppner, R.S. Hartley, M. Miyazono, N.W. Fraser, S. Kesari, V.M.-Y. Lee, Transfectable and transplantable postmitotic human neurons: a potential 'platform' for gene therapy of nervous system diseases, Exp. Neurol. 144 (1997) 92–97.
[114] R. Watanabe, S. Takase-Yoden, T. Ikeda, T. Atsumi, Gene transfer through implantation of embryonal carcinoma cells in the brain, Cell Transplant. 5 (suppl.) (1996) S9–S12.
[115] S.R. Wachtel, C. Bencsics, U.J. Kang, Role of aromatic L-amino acid decarboxylase for dopamine replacement by genetically modified fibroblasts in a rat model of Parkinson's disease, J. Neurochem. 69 (1997) 2055–2063.
[116] M.S. Shoichet, F.T. Gentile, S.R. Winn, The use of polymers in the treatment of neurological disorders, Trends Polym. Sci. 3 (1995) 374–380.
[117] F. Lim, A.M. Sun, Microencapsulated islets as bioartificial endocrine pancreas, Science 210 (1980) 908–910.
[118] C.A. Crooks, J.A. Douglas, R.L. Broughton, M.V. Sefton, Microencapsulation of mammalian cells in a HEMA–MMA copolymer: effects on capsule morphology and permeability, J. Biomed. Mater. Res. 24 (1990) 1241–1262.
[119] P. Aebischer, S.R. Winn, P. Galletti, Transplantation of neural tissue in polymer capsules, Brain Res. 448 (1988) 364–368.
[120] M.S. Shoichet, D.H. Rein, In vivo biostability of a polymeric hollow fiber membrane for cell encapsulation, Biomaterials 17 (1996) 285–290.
[121] M.S. Shoichet, R.H. Li, M.L. White, S.R. Winn, Stability of hydrogels used in cell encapsulation: an in vitro comparison of alginate and agarose, Biotechnol. Bioeng. 50 (1996) 374–381.
[122] M.A. Haralson, Extracellular matrix and growth factors: an integrated interplay controlling tissue repair and progression to disease, Lab. Invest. 69 (1993) 369–372.
[123] P. Aebischer, P.A. Tresco, J. Sagen, S.R. Winn, Transplantation of microencapsulated bovine chromaffin cells reduces lesion-induced rotational asymmetry in rats, Brain Res. 560 (1991) 43–49.
[124] D.F. Emerich, B.R. Frydel, T.R. Flanagan, M. Palmatier, S.R. Winn, L. Christenson, Transplantation of polymer encapsulated PC12 cells: use of chitosan as an immobilization matrix, Cell Transplant. 2 (1993) 241–249.
[125] S.P. Massia, J.A. Hubbell, Covalent surface immobilization of Arg–Gly–Aspand Tyr–Ile–Gly–Ser–Arg-containing peptides to obtain well-defined cell-adhesive substrates, Anal. Biochem. 187 (1990) 292–299.
[126] Y.W. Tong, M.S. Shoichet, Peptide surface modification of poly(tetrafluoroethylene–co-hexafluoroethylene) enhances its interaction with central nervous system neurons, J. Biomed. Mater. Res. 42 (1998) 85–95.
[127] R. Bellamknoda, J.P. Ranieri, P. Aebischer, Laminin oligopeptide derivatized agarose gels allow three-dimensional neurite extension in vitro, J. Neurosci. Res. 41 (1995) 501–509.
[128] P. Tresco, S. Winn, P. Aebischer, Polymer encapsulated neurotransmitter secreting cells:potential treatment for Parkinson's disease, American Society for Artificial Internal Organs 38 (1992) 17–23.
[129] T. Roberts, U. DeBoni, M.V. Sefton, Dopamine secretion by PC12 cells microencapsulated in a hydroxyethyl methacrylate–methyl methacrylate copolymer, Biomaterials 17 (1996) 267–275.
[130] P. Aebischer, M. Goddard, P. Signore, R. Timpson, Functional recovery in hemiparkinsonian primates transplanted with polymer encapsulated PC12 cells, Exp. Neurol. 126 (1994) 1–12.
[131] T. Subramanian, D.F. Emerich, R.A.E. Bakay, J.M. Hoffman, M.M. Goodman, T.M. Shoup, G.W. Miller, A.I. Levey, G.W. Hubert, S. Batchelor, S.R. Winn, J.A. Saydoff, R.L. Watts, Polymer-encapsulated PC12 cells demonstrate high affinity uptake and dopamine in vitro and 18F-dopa uptake and metabolism after intracerebral implantation in nonhuman primates, Cell Transplant 6 (1997) 469–477.
[132] J.H. Kordower, Y.-T. Liu, S.R. Winn, D.F. Emerich, Encapsulated PC12 cell transplants into hemiparkinsonian monkeys: a behavioral, neuroanatomical and neurochemical analysis, Cell Transplant 4 (1995) 155–171.
[133] L.L. Pundt, E.A. Join, J.A. Conrad, W.C. Low, Organization and histochemical phenotype of human fetal cerebellar cells following transplantation into the cerebellum of nude mice, Cell Transplant 6 (1997) 479–489.
[134] H. Bernstein-Goral, B.S. Bregman, Axotomized rubrospinal neurons rescued by fetal spinal cord transplants maintain axon collaterals to rostral CNS targets, Exp. Neurol. 148 (1997) 13–25.
[135] B. Sieradzan, G. Vrbova, Observations on the survival of grafted embryonic motoneurons in the spinal cord of developing rats, Exp. Neurol. 122 (1993) 223–231.
[136] J.P. Hammang, D.R. Archer, I.D. Duncan, Myelination following transplantation of EGF-responsive neural stem cells into a myelin-deficient environment, Exp. Neurol. 147 (1997) 84–95.
[137] J.T. Buchanan, H.O. Nornes, Transplants of embryonic brainstem containing the locus coeruleus into spinal cord enhance the hindlimb flexion reflex in adult rats, Brain Res. 381 (1986) 225–236.

[138] J.W. Commissiong, Y. Sauve, The physiological basis of transplantation of fetal catecholaminergic neurons in the transected spinal cord of the rat, Comp. Biochem. Physiol. 93A (1989) 301–307.
[139] D.F. Emerich, S.R. Winn, J. Harper, J.P. Hammang, E.E. Baetge, J.H. Kordower, Implants of polymer-encapsulated human NGF-secreting cells in the nonhuman primate: rescue and sprouting of degenerating cholinergic basal forebrain neurons, J. Comp. Neurol. 349 (1994b) 148–164.
[140] M.F. Beal, N.W. Kowall, D.W. Ellison, M.F. Mazurek, K.J. Swartz, J.B. Martin, Replication of the neurochemical characteristics Huntington's disease by quinolinic acid, Nature 321 (1986) 168–171.
[141] D.F. Emerich, M.D. Lindner, S.R. Winn, E.-Y. Chen, B.R. Frydel, J.H. Kordower, Implants of encapsulated human CNTF-producing fibroblasts prevent behavioral deficits and striatal degeneration in a rodent model of Huntington's disease, J. Neurosci. 16 (1996) 5168–5181.
[142] M.S. Shoichet, S.R. Winn, S. Athavale, J.M. Harris, F.T. Gentile, Poly(ethylene oxide)-grafted thermoplastic membranes for use as cellular hybrid bioartificial organs in the central nervous system, Biotechnol. Bioeng. 43 (1994) 563–572.
[143] S.R. Winn, B. Zielinski, P.A. Tresco, A.P. Signore, C.B. Jaeger, L.A. Greene, P. Aebischer, Behavioral recovery following intrastriatal implantation of microencapsulated PC12 cells, Exp. Neurol. 113 (1991) 322–329.
[144] P.A. Tresco, S.R. Winn, C.B. Jaeger, L.A. Greene, P. Aebischer, Polymer-encapsulated PC12 cells: longterm survival and associated reduction in lesioned-induced rotational behavior, Cell Transplant. 1 (1992) 255–264.
[145] M.D. Lindner, D.F. Emerich, Therapeutic potential of a polymer-encapsulated L-DOPA and dopamine-producing cell line in rodent and primate models of Parkinson's disease, Cell Transplant 7 (1998) 165–174.
[146] M.D. Lindner, M.A. Plone, T.D. Mullins, S.R. Winn, S.E. Chandonait, J.A. Stott, T.J. Blaney, S.S. Sherman, D.F. Emerich, Somatic delivery of catecholamines in the striatum attenuate Parkinsonian symptoms and widen the therapeutic window of oral Sinemet in rats, Exp. Neurol. 145 (1997) 130–140.
[147] S.R. Winn, M.D. Lindner, A. Lee, G. Haggett, J.M. Francis, D.F. Emerich, Polymer-encapsulated genetically modified cells continue to secrete human nerve growth factor for over one year in rat ventricles: behavioral and anatomical consequences, Exp. Neurol. 140 (1996) 126–138.
[148] F.H. Gage, Cell therapy, Nature 392 (suppl.) (1998) 18–24.