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Review

Delivery systems for small molecule drugs, proteins, and DNA: the neuroscience/biomaterial interface

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Manipulation of cellular processes in vivo by the delivery of drugs, proteins or DNA is of paramount importance to neuroscience research. Methods for the presentation of these molecules vary widely, including direct injection (either systemic or stereotactic), osmotic pump-mediated chronic delivery, or even implantation of cells engineered to indefinitely secrete a factor of interest. Biomaterial-based delivery systems represent an alternative to more traditional approaches, with the possibility of increased efficacy. Drug-releasing biomaterials, either as injectable microspheres or as three-dimensional implants, can deliver a molecule of interest (including small molecule drugs, biologically active proteins, or DNA) over a more prolonged period of time than by standard bolus injection, avoiding the need for repeated administration. Furthermore, sustained-release systems can maintain therapeutic concentrations at a target site, thus reducing the chance for toxicity. This review summarizes applications of polymer-based delivery of small molecule drugs, proteins, and DNA specifically relevant to neuroscience research. We detail the fabrication procedures for the polymeric systems and their utility in various experimental models. The biomaterial field offers unique experimental tools with downstream clinical application for the study and treatment of neurologic disease. D 2004 Elsevier Inc. All rights reserved.

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Introduction

Biomaterials are increasingly employed for sustained delivery applications to enhance drug safety and efficacy and to improve patient compliance. A number of drug-releasing polymer products have been approved for clinical use (see Table 1), such as the Gliadel wafer which releases carmustine for localized treatment of glioblastoma multiforme in the brain (Brem and Gabikian, 2001). The number of products that combine a drug with a polymer or device is expected to increase significantly, which was a factor that motivated the recent establishment of the Office of Combination Products by the Food and Drug Administration. A variety of therapeutic agents can be encapsulated into polymeric release systems, including small molecule drugs, proteins, or even DNA encoding a protein of interest (Richardson et al., 2001a). Historically, the drug delivery field has focused on the release of small molecule drugs for the treatment of disease. More recently, however, methodologies have been developed to release proteins and DNA from biomaterials, which expands the opportunities to direct physiological processes. This review provides background regarding the materials commonly utilized in sustained delivery and examines their applications relevant to neuroscience.

Material properties

Biomaterials have been successfully employed in neuroscience for numerous drug delivery applications (Langer,

Abbreviations: EVAc, polyethylene-co-vinyl acetate; pCPP:SA, poly[bis(*p*-carboxyphenoxy)propane:sebacic acid; PEG, polyethylene glycol; PLA, polylactide; PLGA, poly(lactide-co-glycolide); PVA, polyvinyl alcohol.

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Table 1
FDA-approved polymer-based drug delivery systems

Product name	Material	Drug released	Approved	Application
Injectable format				
Lupron depot	PLA	leuprolide acetate	1995	prostate cancer, endometriosis
Nutropin depot	PLGA	human growth hormone	1999	growth failure
Trelstar depot	PLGA	triptorelin pamoate	2000	Prostate cancer
Implants				
Cypher coronary stent	EVAc + PBMA	sirolimus	2003	coronary artery disease
Gliadel wafer	pCPP:SA	carmustine	1996	glioblastoma multiforme
Taxus coronary stent	translute[a]	paclitaxel	2003	coronary artery disease
Zoladex	PLA	goserelin acetate	2002	prostate cancer, endometriosis

[a] A Boston Scientific proprietary nondegradable polymer.

1998). The materials utilized have included both natural polymers (e.g., collagen) as well as synthetics [e.g., silicone, poly-lactide-co-glycolide (PLGA), and polyethylene vinyl-co-acetate (EVAc)]. Hydrophilic polymers, such as collagen, fibrin, and alginate, can be used to fabricate matrices that are extensively hydrated, called hydrogels. Bioactive molecules incorporated into hydrogels are released primarily by diffusion at a rate inversely proportional to the size of the drug, providing little control over the rate of release (Gombotz and Pettit, 1995). The limited ability to control release rates from hydrogels led to the use of synthetic polymers as sustained release vehicles. Release from the nondegradable synthetic polymer EVAc is also governed by diffusion through the material (Maurin et al., 1992), although altering the ratio of the polymer components can change the pore structure and therefore the release rate. Synthetic degradable polymers, such as the polyester PLGA, have release profiles that are dependent upon diffusion and pore structure, as well as degradation of the material itself (Gombotz and Pettit, 1995; Langer, 1990), providing an additional level of control over the rate of release of the incorporated molecule of interest.

Biodegradable polymers are highly desirable for drug delivery applications, since the device will disappear over time, eliminating the need for a second surgical procedure to retrieve the device. PLGA is perhaps the most widely used biopolymer, its prevalence due to its commercial availability, controllable degradation rate (Lu et al., 2000a), proven biocompatibility (Athanasiou et al., 1996), and FDA approval, having been used as a resorbable suture material for decades (Horton et al., 1974). Degradation of PLGA occurs by hydrolysis, the rate of which is largely dependent upon the molecular weight and the ratio of the copolymer components lactide and glycolide. Many different formulations of PLGA are available, with degradation times ranging from a few weeks to many months (Holy et al., 1999; Lu et al., 2000a; Visscher et al., 1985). Thus, PLGA with an appropriate composition and molecular weight can be selected that provides the desired degradation properties. Furthermore, PLGA has been specifically shown to be biocompatible with the nervous system. PLGA microspheres stereotactically injected into the striatum elicited only a small immune response, which was similar to injection of vehicle alone, with no direct evidence of toxicity to neural tissue (Emerich et al., 1999; Menei et al., 1993). These properties make PLGA an excellent choice for sustained delivery applications to neuroscience. Another class of degradable polymers, polyanhydrides, share some of these desirable properties, with the primary difference being the manner in which they degrade (Dondrow, 1991). However, polyanhydrides are not currently commercially available, thereby limiting their widespread application.

Delivery systems

Polymeric delivery systems have the potential to (i) maintain therapeutic levels of a drug, (ii) reduce harmful side effects, (iii) decrease the amount of the molecule required, (iv) decrease the number of dosages, and (v) facilitate the delivery of drugs with short in vivo half-lives (Langer, 1998). Currently, the most common method to deliver therapeutic factors to the CNS involves surgically implanting pump or cannula systems (Harbaugh et al., 1988). However, pumps frequently become clogged within a few days, which limits their ability to sustain effective concentrations (Jones and Tuszynski, 2001). Furthermore, the molecule is maintained in an aqueous reservoir, which is not suitable for therapeutic agents with short half-lives. Polymeric encapsulation can protect the incorporated drug from degradation by the surrounding aqueous environment (Schwendeman, 2002). The selection of the appropriate format, microspheres versus a three-dimensional implant, will depend upon the intended application as well as the molecule to be delivered.

Microspheres

Polymer microspheres, which can range in diameter from one to several hundred micrometers, loaded with a molecule of interest provide flexibility to the method and site of administration. As sustained delivery vehicles, microspheres can provide localized release of a therapeutic factor (Putney and Burke, 1998) and have already been employed in

diverse applications within the central nervous system (CNS) (Menei et al., 1994). The administration of drugs to the CNS is a significant challenge, as many drugs will not cross the blood-brain barrier (BBB). An appealing aspect of a microsphere-based delivery system is that stereotactic injection to a specific region of the brain bypasses the BBB, thereby enabling sustained delivery directly to the CNS of a known dosage.

While injectable microspheres are an appealing delivery format, the process of efficiently encapsulating the molecule of interest into microspheres is technically challenging. The release rate of a bioactive molecule from polymer microspheres depends upon the relative ratio of surface-associated to encapsulated drug. The process of microsphere formation invariably leaves some of the incorporated molecule at the microsphere surface. This surface-associated factor is released rapidly at physiological conditions and is called the bburst releaseQ (Huang and Brazel, 2001). The initial burst is followed by a more prolonged release of the remaining content of the microspheres, with rates largely dictated by the properties of the polymer employed. While such a biphasic release profile may be desirable for some applications, the unpredictable nature of the initial burst has led to the development of new methods to produce a more linear rate of release, such as the fabrication of double-walled microspheres (Lee et al., 2002; Pekarek et al., 1994; Shi et al., 2003; Yang et al., 2003). Importantly, note that release rates from polymeric systems are typically determined by in vitro assay, which can be significantly different from the release in vivo.

Implants

Three-dimensional implants, which can be on the millimeter to centimeter scale, can provide a larger depot of the bioactive factor, yet typically require a more invasive procedure. Implants are much easier to fabricate than drug-loaded microspheres, typically employing a solvent casting method (see Materials processing). This method has the advantages of using a simpler process to optimize and larger physical size enabling greater quantities of the molecule to be incorporated. The major limitation of this method is the types of molecules that can be incorporated, due to the direct contact with an organic solvent that can compromise the integrity and activity of the molecule.

These implants can also be fabricated into structural supports (Hudson et al., 2000; Teng et al., 2002), which can be used to promote nerve regeneration (Evans et al., 1999; Oudega et al., 2001) or as vehicles for cell transplantation (Teng et al., 2002). The geometry of the structure (e.g., shape, size, porosity) can be tailored to the application. The porosity of the scaffold is an important design parameter, which influences cell seeding or cell infiltration from the surrounding tissue. Additionally, the fabrication can be designed to incorporate extracellular matrix proteins that will influence cell adhesion and migration in the scaffold. These structures can be formed by direct casting of the polymer and drug into a desired shape, or by processing microspheres loaded with the molecule of interest into a three-dimensional structure (Harris et al., 1998). The structural features of the scaffold can function in cell organization, whereas the release of the incorporated drug can regulate the local biochemical environment (Murphy and Mooney, 1999; Shea et al., 1999). For example, in peripheral nerve regeneration, a nerve conduit releasing neurotrophic factors can stimulate neuron survival and axon growth, while ensuring that regenerating axons are directed to the distal stump.

Materials processing

Emulsion-based methods are the predominant approach for fabricating drug-releasing microspheres from synthetic polymers, although a variety of methods are available (Watts et al., 1990). The selection of an encapsulation method depends upon several factors, including the polymer and the solubility and stability of the molecule to be incorporated. Organic solvents are used to dissolve the polymer, which can affect the activity of the encapsulated molecule. If the molecule of interest is either soluble in an organic solvent (Birnbaum et al., 2000) or is stable in a crystalline form when dispersed in an organic solvent (Ando et al., 1999; Gombotz et al., 1991), the encapsulation can be accomplished using a water/oil single emulsion method (Fig. 1). However, water-soluble molecules may be inacti- vated by direct exposure to the solvent and are processed with a water–oil–water double emulsion method (Cohen et al., 1991) (Fig. 2). While the double emulsion method is more technically challenging and requires careful optimization, it significantly diversifies the range of bioactive molecules that can be incorporated into polymer delivery systems. A significant challenge to the emulsion method lies in the encapsulation efficiency. Solution concentrations, loading ratio, and the method and extent of emulsification must be optimized; otherwise, a large percentage of the molecule to be encapsulated can be lost to the emulsion stabilizer solution.

Three-dimensional implants capable of controlled release can be fabricated using emulsion-based methods; however, numerous techniques are under development to enable the fabrication of more complex geometries. Rods, tubes, and similar structures can be fabricated with a solvent-casting approach (Rhine et al., 1980). This method is similar to the single emulsion method for microsphere production, and also requires that the molecule of interest is either soluble in the organic polymer solution or stable as a crystalline solid when dispersed in the polymer solution. Alternatively, hydrophilic compounds have been incorporated intro structural scaffolds using a carbon dioxide foaming process (Harris et al., 1998). In this approach, lyophilized compounds are mixed with polymer microspheres or the

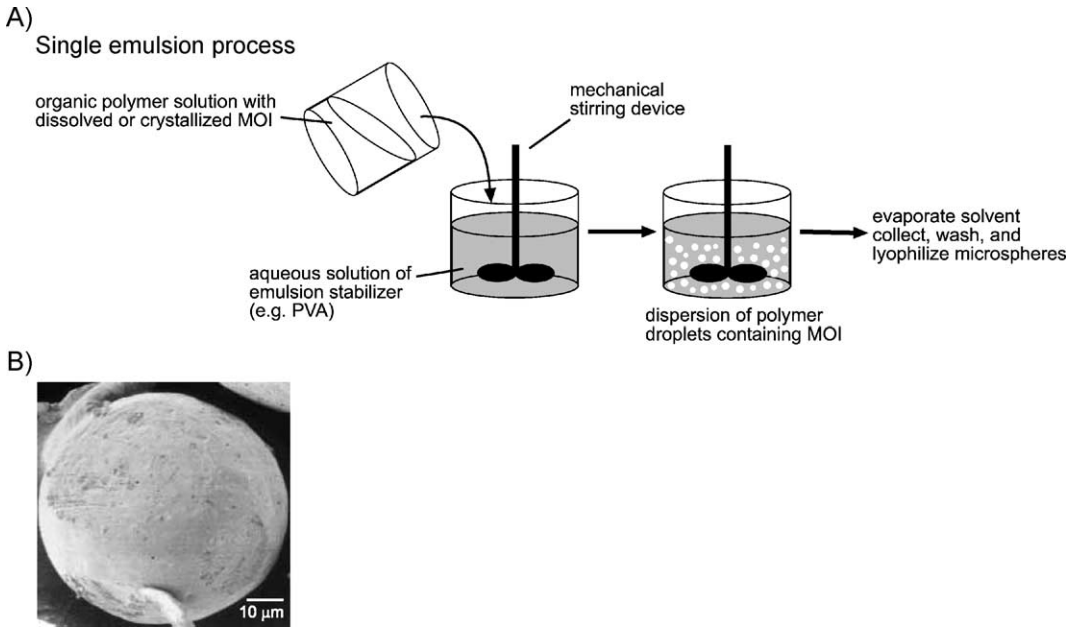

Fig. 1. (A) Water/oil single emulsion for encapsulation of organic-soluble or crystallized molecule of interest (MOI). MOI is either dissolved or dispersed (if in crystallized form) in an organic polymer solution. The organic solution is emulsified with a stabilizer, such as polyvinyl alcohol (PVA), which prevents the organic droplets from coalescing. The speed and duration of agitation with a mechanical stirring device will dictate the size of the droplets formed, with stronger agitation breaking up the polymer into smaller droplets. Solvent evaporation yields MOI-loaded microspheres dispersed in the PVA solution, which are collected and washed by centrifugation or filtration, and lyophilized. (B) Representative SEM of a PLGA microsphere made by single emulsion.

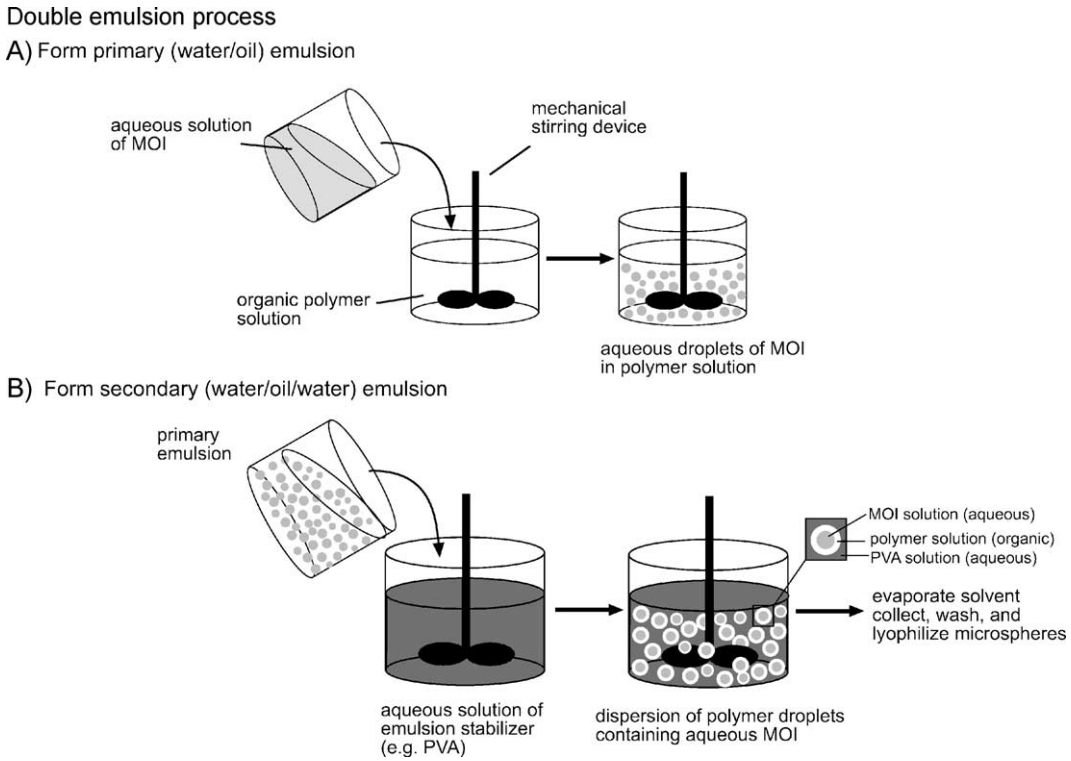

Fig. 2. Water/oil/water double-emulsion method for encapsulation of hydrophilic MOIs in polymer microspheres. (A) An aqueous solution of the MOI is emulsified in an organic polymer solution, forming the primary (water/oil) emulsion. (B) The primary emulsion is then introduced into an aqueous solution with a stabilizer (e.g., PVA) and emulsified, forming the secondary (water/oil/water) emulsion. As in the single emulsion method, the speed and duration of agitation with a mechanical stirring device control the size of the droplets formed during each of the emulsifications. Solvent evaporation yields MOI-loaded microspheres dispersed in the PVA solution, which are collected and washed by centrifugation or filtration, and lyophilized.

emulsion-based methods are employed to encapsulate hydrophilic molecules into microspheres, which are then processed into the desired structure. Methods are being developed to allow molecular-level design of polymer scaffolds, which have the desired geometry and provide precise control over the release of one or more factors.

Small molecule drug delivery

The delivery of small molecule drugs from a sustained release polymer formulation has broad applications to neuroscience research, including the treatment of nervous system tumors and delivery of neurotransmitters. Pharmacologic approaches are commonly employed, and biomaterial-mediated delivery schemes offer a unique method to deliver these drugs. A diverse array of small molecules have been successfully incorporated into polymeric systems (Table 2), optimistically suggesting that the delivery of many other small molecules is also possible. Potential drugs of interest which are applicable to biomaterial-based systems include neural tracers (i.e., DiI or Fluorogold) or agonists or antagonists of enzymes or ion channels, to name a few.

Sustained delivery from biomaterial-based systems can be used both to model as well as to treat disease. Haik et al. (2000) used EVAc implants in lieu of an osmotic pump to model Huntington's disease. Osmotic pumps used to chronically administer quinolinic acid (QA) mimic the progressive nature of Huntington's disease more accurately than acute administration (Bazzett et al., 1993; Marrannes

Table 2
Small molecule drugs released from biomaterials

Drug	Format	Application	Observations	Reference
Bromodeoxyuridine	PLGA microspheres	in vivo cell labeling	efficient labeling at cortical lesion site	(Maysinger et al., 1994)
5-Fluorouracil	PLGA microspheres	in vitro release	release for up to 21 days	(Boisdron-Celle et al., 1995; Yeh et al., 2001)
		striatal glioma	increase in animal survival correlated with release rate	(Menei et al., 1996; Roullin et al., 2003)
Iododeoxyuridine	PLGA microspheres	in vitro release	release over 7–35 days	(Reza and Whateley, 1998)
Dopamine, norepinephrine	PLGA microspheres	Parkinsonian rat model	reduction in rotational behavior for up to 8 weeks	(McRae and Dahlstrom, 1994; McRae et al., 1994)
Retinoic acid	PLGA microspheres	in vitro release; in vivo treatment of vitreoretinopathy model	release for up to 40 days; reduction in retinal detachment for up to 40 days	(Giordano et al., 1993)
Paclitaxel	PLGA microspheres	in vitro release	release over 60 days	(Feng et al., 2000)
	PEG-PLA microspheres	in vivo intravenous release	higher drug tissue levels than seen with direct injection	(Kim et al., 2001)
Carboplatin	PLGA microspheres	in vitro release	release over 22 days	(Chen and Lu, 1999)
	PLGA microspheres	in vivo intracerebral release	linear release over 30 days	(Chen et al., 1997)
Isoniazid	PLGA microspheres	in vitro release; in vivo subcutaneous release	released over 49 days; detectable in plasma for 49 days	(Dutt and Khuller, 2001)
Estradiol	PLGA microspheres	in vitro release	release over 40–60 days	(Birnbaum et al., 2000)
Dopamine	EVAc implant	in vitro release	release for 15–30 days	(Freese et al., 1989; Winn et al., 1989)
		in vivo stratium release	high levels of striatal dopamine for up to 60 days	(During et al., 1989)
		Parkinsonian rat model	reduction in rotational behavior for 2 weeks	(Winn et al., 1989)
Levodopa	EVAc implant	in vitro release	release up to 2 years	(Sabel et al., 1990)
Adenosine	EVAc implant	epileptic rat model	reduction in seizure activity for up to 2 weeks	(Boison et al., 1999)
Substance P	EVAc implant	rat model of excitotoxicity	prevented loss of striatal dopaminergic neurons after QA administration	(Sanberg et al., 1993)
Carmustine	pCPP:SA implant	in vivo release in brain parenchema	increased drug persistence over direct stereotactic injection	(Grossman et al., 1992)
Hormone agonist	PLGA implant	in vivo subcutaneous release	release over 20–50 days	(Heinrich et al., 1991)

and Wauquier, 1988; Sanberg et al., 1989; Shear et al., 1998). Replacement of the osmotic pump with a QA-releasing EVAc implant produced similar neuroanatomical and behavioral deficiencies (Haik et al., 2000). Alternatively, dopamine has been delivered to the striatum for the treatment of Parkinson's disease. Continuous delivery of the dopamine precursor levodopa reduces the variability in responsiveness to treatment commonly seen with more pulsatile delivery schemes, which is thought to be linked to fluctuations in serum levels of the drug (Nutt et al., 2000; Obeso et al., 1994). Neurotransmitter-releasing PLGA microspheres applied to treat a Parkinsonian rat model reduce rotational behavior in a manner that is dependent upon the degradation rate of the polymer used (McRae and Dahlstrom, 1994; McRae et al., 1994). The development of delivery systems capable of steady drug release for periods of years to treat a slow, progressive disease such as Parkinson's is challenging. These studies indicate that polymer-mediated delivery can effectively administer small molecule drugs for extended periods of time in vivo, performing similarly to osmotic pump systems. Release rates can be tailored to the application, but the desired duration of release must be considered within the current limitations of biomaterial systems, which can deliver incorporated drugs over periods ranging from weeks to years, depending upon the molecule and polymer formulation.

Drug-releasing biomaterials enable localized delivery to a target tissue, which can avoid the toxicity associated with the systemic administration of some compounds. Small molecule drugs are efficacious on numerous cell types; thus, systemic delivery can result in drug activity within non-target tissue. Furthermore, some drugs interact with families of proteins, affecting multiple cellular processes, which complicates experimental and therapeutic applications. Since many neuronal functions and, therefore drug sensitivities, are regional and cell type specific, localized drug release systems would significantly advance the study and treatment of neurologic diseases. Implantation of Gliadel wafers following tumor resection for sustained, localized release of carmustine improves patient survival (Brem and Gabikian, 2001; Brem et al., 1991, 1995a,b). Antimetabolic agents are toxic to actively dividing cells; thus, spatially localized delivery at the tumor site reduces exposure to healthy cells. Additionally, carmustine is rapidly degraded under physiological conditions (Loo et al., 1966), and would otherwise require complex administration regimens. Other antimetabolic agents, such as the chemotherapy drug 5-fluorouracil (5-FU), are excellent candidates for polymer-mediated release.

Numerous types of small molecule drugs have been delivered from polymeric systems (Table 2), many of which can be tailored to neuroscience applications. For example, delivery of nucleotides and nucleosides would have numerous applications, given their roles in the nervous system (Rathbone et al., 1999). Bromodeoxyuridine (BrdU), a thymidine analog, is commonly used experimentally to monitor cell proliferation and migration (Dolbeare, 1995) and used clinically as a radiosensitizer for the treatment of tumors in the nervous system (Freese et al., 1994). When used in the CNS, it is often delivered by multiple injections or by osmotic pump (Dolbeare, 1996), for which BrdU-releasing microspheres would be an ideal alternative. Other candidates include dibutyryl- cAMP, which has been used in vivo to improve regeneration in the presence of inhibitory molecules (Qiu et al., 2002). Adenosine can serve as an endogenous anticonvulsant (Dragunow and Faull, 1988; Dragunow et al., 1985), promote myelination (Stevens et al., 2002), and function in a number of other neuromodulatory roles (Rathbone et al., 1999). Adenosine has been successfully released from three-dimensional polymeric implants in vivo (Rathbone et al., 1999) and inosine was released from a polymer guidance tube to improve nerve regeneration (Hadlock et al., 1999), providing proof of concept that release of purines from a polymer matrix is possible. Alternatively, retinoic acid (RA)-releasing microspheres (Giordano et al., 1993) have a variety of possible neural applications, such as developmental studies. RA is important in a number of aspects of embryonic develop- ment, including patterning of the neural crest and neurulation (Morriss-Kay and Sokolova, 1996). Conse- quently, RA treatment is used experimentally in neuro- science paradigms, such as the differentiation of neurons from embryonic stem cells (Guan et al., 2001), for which the ability to release RA in vivo over a prolonged period of time may be desirable.

Commercially available degradable drug-releasing polymer pellets may present an appealing option for some investigators as a ready source for use in pilot studies. A wide array of small molecule drugs, such as catecholamines and nucleosides, incorporated into timed-release biodegradable pellets (Innovative Research of America, Sarasota, FL) can be purchased for use in animal models. Offerings include proprietary polymer formulations that provide a variety of release rates and dosages. While limitations associated with the proprietary nature of these pellets may restrict control over the physical properties of the implant and drug release rate from it, this may be adequate for preliminary experiments, before developing a formulation tailored to the specific application.

Protein delivery

Growth factors, monoclonal antibodies, matrix-degrading enzymes, and various other proteins play significant roles in neural development and function, with the potential to improve current therapies. Proteins are primarily responsible for performing or directing cellular functions, and their presence at the appropriate concentration, time, and location is critical for normal development and function of the nervous system. Neurotrophic factors bind to cell

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