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Nerve growth factor delivery systems

Michael F. Haller, W. Mark Saltzman*

School of Chemical Engineering, Cornell University, Ithaca, NY 14853, USA

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Abstract

Growth factors encourage tissue regeneration and differentiation, accelerate wound healing, and modulate neural repair. Thus, growth factor administration may become a useful treatment for neurodegenerative diseases, such as Alzheimer's disease or Parkinson's disease, which are characterized by the degeneration of neuronal cell populations. Controlled-release polymer delivery systems may be an important technology in enabling the prevention of neuronal degeneration, or even the stimulation of neuronal regeneration, by providing a sustained release of growth factors to promote the long-term survival of endogenous or transplanted cells. © 1998 Elsevier Science B.V.

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1. Introduction

Molecules which encourage tissue regeneration and differentiation are generally classified as 'growth factors.' These soluble signalling proteins represent several distinct structural families of molecules; each growth factor generally possesses a variety of biological activities. In the developing embryo, growth factors called morphogens signal cells to proliferate and differentiate to form specialized tissues such as embryonic mouse forelimbs and Xenopus vasculature, as recently reviewed [1,2]. In *Drosophila* embryos, a gradient of the *bicoid* protein is essential for the formation of anterior structures [3], while other proteins are essential for formation of posterior, dorsal, and ventral structures [4]. Growth factors, such as epidermal growth factor (EGF), promote early eyelid opening in mice, as well as skin and lung development in fetal lambs [5]. The study of expression and action of various growth factors has become an important subfield of developmental biology.

In some cases, growth factors can accelerate cellular proliferation and differentiation in adults, offering the possibility that growth factors may have important roles in therapy as well. This aspect of growth factor biology is best exemplified by the activity of molecules on accelerated wound healing and neural repair. Transforming growth factor a (TGF-α) is a potent epithelial cell mitogen, which accelerates mid-dermal wound healing [6]. Also, basic fibroblast growth factor (bFGF) and platelet derived growth factor (PDGF) induce accelerated healing of chronic gastric ulcers, chronic erosive gastritis, and ulcerative colitis [7]. Administration of nerve growth factor (NGF) promotes the survival and neurite outgrowth of degenerating cholinergic

*Corresponding author. Tel.: +607 2552657; fax: +607 2551136; e-mail: saltzman@cheme.cornell.edu

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neurons [8,9]; degeneration of specific cell populations is a frequent finding in disorders of the central nervous system such as Alzheimer's disease, Parkinson's disease, and Huntington's chorea. In addition, continuous infusions of EGF into adult animals increases the proliferation of neural precursor cells [10], suggesting a possible treatment for several neural disorders.

One potential undesirable effect of growth factors stimulation of cell proliferation is that they may cause uncontrolled cell growth, as occurs in cancer. EGF and TGF-α, and their receptors, are often overexpressed in squamous carcinomas, gliomas, and other tumor types [11–14], as compared to normal tissues, leading to autocrine loops [15–17] and uncontrolled proliferation. It may be possible, however, to harness this characteristic of tumor biology to develop new therapies based on covalently linkage of a cellular toxin [18–20] to these overexpressed growth factor ligands. Alternatively, cancer therapies might involve the delivery of antibodies to ligands or overexpressed receptors [21] in an effort to reduce proliferation, as reviewed elsewhere [5].

Modern biotechnology permits the large-scale production of highly purified proteins; therefore, large quantities of growth factors can now be manufactured for use in humans. But growth factors can be exceedingly difficult to administer to patients, since they typically have half-lives only on the order of minutes [22–24]. These factors are also macromolecules, so they slowly penetrate tissue barriers, such as capillary walls. In addition, as illustrated above, growth factors often possess biological activity at multiple tissue sites throughout the body; therefore, systemic administration can lead to toxicity. In view of these difficulties, new methods for growth factor delivery are needed. The most promising new methods involve polymeric controlled-release devices, which can be engineered to provide precisely controlled, prolonged growth factor delivery at a localized site. In this review, these methods are illustrated using our own experience with NGF delivery to the nervous system as an example.

2. Polymers for controlled release of growth factors

We have been studying an approach for the delivery of agents to the brain, in which agents are encapsulated in polymer pellets or microspheres and then implanted directly into the brain tissue at the site in need of treatment [25–29]. This approach has been tested clinically for the treatment of brain tumors [30]. With the development of controlled release methods for labile macromolecules, this approach can also provide continuous, high-dose delivery of NGF [31,32].

When agents are directly administered to the brain from an implanted polymer, their concentrations in brain tissues decrease exponentially with distance away from the polymer (Fig. 1), suggesting a tissue transport mechanism consisting of diffusion and elimination. For most compounds, the majority of the agent is localized within a 1–2 mm region near the surface of the polymer [26,33]. We believe that this occurs because molecules released from the polymer are eliminated from the brain before they can penetrate sufficiently into the tissue. Design of polymers that release larger quantities of agent does not solve this problem [25], so we have synthesized compounds that are more slowly eliminated from the central nervous system. In one approach, active compounds were conjugated to the polysaccharide dextran, which is eliminated slowly from the brain [34], probably due to inefficient degradation by lysosomes [19]. Dextran–methotrexate conjugates penetrate three times farther in tumors, with no loss of tumor-killing activity [35]; dextran–NGF conjugates also penetrate significantly farther in brain tissue, with an apparent rate of elimination ~seven times lower than unmodified NGF [36].

The approach described above results in transport of the agent to a distance determined largely by the relative rates of drug elimination and diffusion. In the future, as more is learned about the mechanisms of protein transport and metabolism in complex cell communities, polymer delivery systems can be designed to deliver agents in effective concentrations to regions far from the source, possibly leading to more efficacious cholinergic neuron survival or brain tumor therapy.

3. Polymers for growth factor delivery to cell transplants

Biomaterials may also be useful in cell trans-

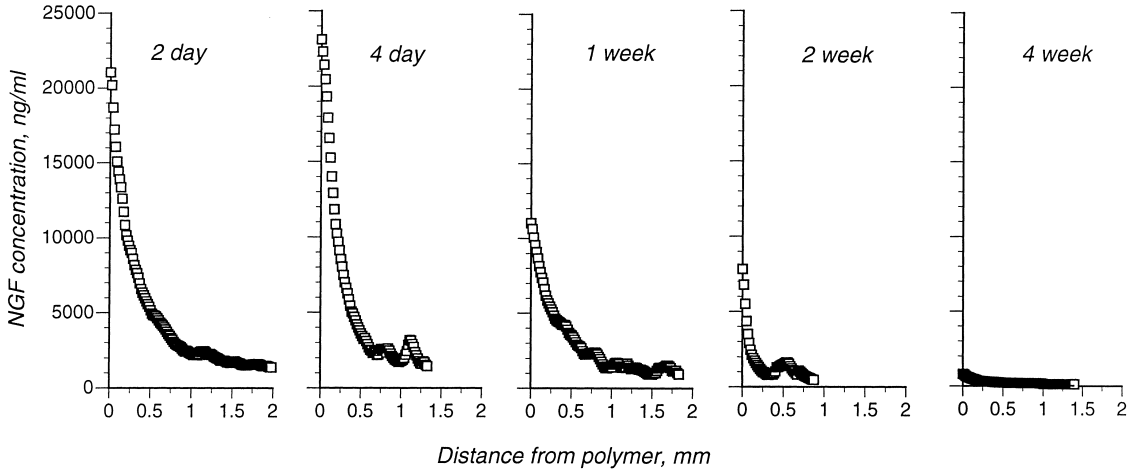

Fig. 1. Concentration profiles in the vicinity of a polymer implant containing [^{125}I]NGF at 2, 4, 7, 14, and 28 days. Individual rat coronal sections were exposed to autoradiographic film, which was developed, scanned, and digitized. The concentration of NGF was determined by comparison of sample intensity with intensity of standards of known activity. Reproduced from [33], with permission.

plantation by providing opportunities to supplement transplanted tissue with controlled quantities of growth factors. Clinical research scientists have been testing the idea that brain cell transplants can restore lost brain function in patients with Parkinson's disease [37–41] and Alzheimer's disease [42]. Two alternate approaches have been used: transplantation of brain tissue segments and injection of cell suspensions created by dissociating brain tissue segments. Each approach has distinct advantages and disadvantages. Whole tissue segments provide intact tissue with functional cell–cell contacts; however, tissue segments are difficult to vascularize and must be placed in a cavity within the host brain, disrupting existing neural structures. Cell suspensions are relatively easy to transplant at any brain location since they are simply injected, but the suspensions have reduced function, possibly due to disruption of their intimate contact with one another. We have been studying an alternate method that might provide advantages of both whole tissue and cell suspensions. In this approach, cell suspensions are created by dissociating brain tissue segments; the suspended cells are then reaggregated into small, tissue-like units, which are still small enough to be injected [43].

In one example of this approach, fetal cell suspensions that were introduced simultaneously with an NGF-releasing polymer had increased activity of choline acetyltransferase (ChAT), an enzyme indicative of cholinergic cell activity, (Fig. 2) [43]. The effect was relatively short-lived, however, as evidenced by the decrease in ChAT activity at two months after transplantation. The decrease in ChAT activity coincided with the decrease in NGF delivery; after four weeks, almost all of the NGF had been exhausted (Fig. 1). With longer-lived conjugates, it may be possible to extend the period of increased ChAT activity for months or years.

Supply of growth factors appears to be particularly important for enhancing cell survival and function in the period after transplantation. Another method to provide long-term delivery of growth factors is to immobilize cell growth factors on polymers [44]. NGF [45] and EGF [46] remain biologically active when chemically immobilized to a surface. There is good evidence that immobilized growth factors accelerate cell growth much more potently than free growth factors [47], possibly due to multivalent simultaneous stimulation and decreased down-regulation of receptors; this concept is reviewed elsewhere [48].

In addition to polymer-based delivery systems, another approach to providing extended delivery of growth factors is by using genetically-engineered cells [49]. We have studied one particular fibroblast cell line [50] that was genetically engineered to produce NGF. Aggregates of fibroblasts produced

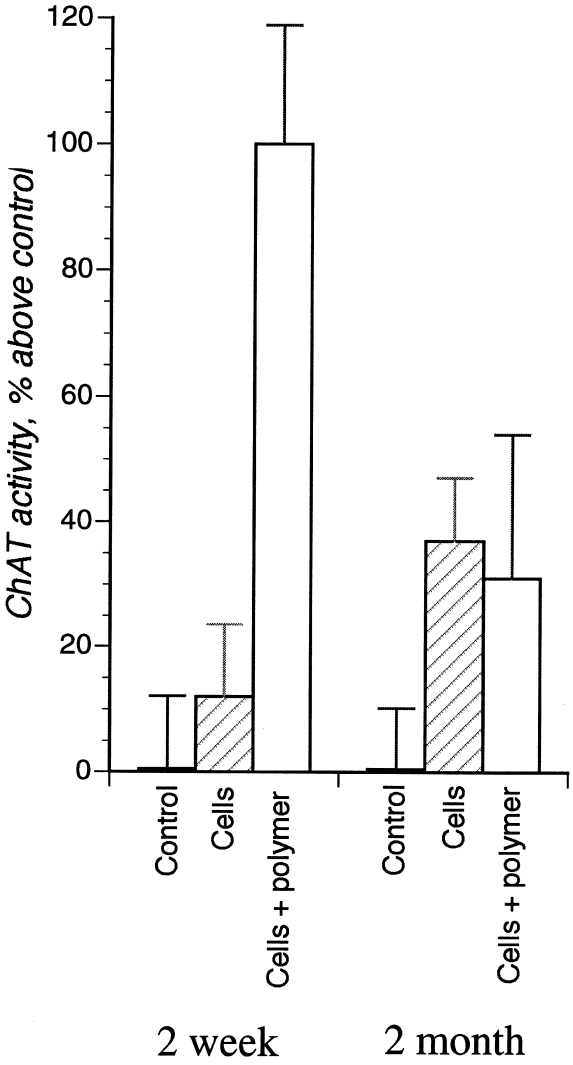

Fig. 2. Choline acetyltransferase (ChAT) activity in brain sections containing the septal transplant site of (a) control, (b) transplants of fetal cell suspensions, or (c) transplants of fetal cell suspensions and an NGF-releasing polymer. Bars indicate the mean value for three identically treated animals; error bars indicate standard deviations.

more NGF than non-aggregated cells [51], suggesting that a key element in enhancing transplanted cell viability and function is the ability to control cell–cell contact. To achieve this end, we have designed polymer–peptide conjugates that serve as molecular seeds for cell aggregation [52]. Conjugate concentration, molecular weight, peptide content, and degree of substitution can all be used to control the properties of the resulting aggregates [53]. These cell aggregation techniques can be adapted to permit the co-aggregation of cells of different types, so that neurons and growth factor-producing cells can be delivered together in engineered tissue aggregates.

4. Conclusion

Biocompatible polymers can be used to produce clinically important delivery systems for the brain. Evidence already collected using animals models suggests that these agents and delivery systems will be therapeutic for many diseases, most notably Alzheimer's disease and Parkinson's disease. As this technology expands in the future, polymers may become an essential ingredient in transplantation surgery, by releasing proteins that enhance cell survival or providing signals for cell assembly into tissues. It is interesting to note that implanted controlled-release polymers result in a concentration gradient nearly identical to the one that defines the organizational pattern in Drosophila embryos [54]. Thus it may be possible to deliver controlled gradients of morphogens to aid in forming elaborate tissue structures in the near future.

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