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A glial cell line-derived neurotrophic factor delivery system enhances nerve regeneration across acellular nerve allografts

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ABSTRACT

Acellular nerve allografts (ANAs) are used clinically to bridge nerve gaps but these grafts, lacking Schwann cells and therapeutic levels of neurotrophic factors, do not support regeneration to the same extent as autografts. Here we investigated a local drug delivery system (DDS) for glial cell line-derived neurotrophic factor (GDNF) controlled release to implanted ANAs in rats using drug-loaded polymeric microspheres (MSs) embedded in a fibrin gel. In a rat hindlimb nerve gap model, a 10 mm ANA was used to bridge a 5 mm common peroneal (CP) nerve gap. Experimental groups received DDS treatment at both suture sites of the allografts releasing GDNF for either 2 weeks or 4 weeks. In negative control groups, rats received no DDS treatment or empty DDS. Rats receiving nerve isografts served as the positive control group. The numbers of motor and sensory neurons that regenerated their axons in all the groups with GDNF MS and isograft treatment were indistinguishable and significantly higher as compared to the negative control groups. Nerve histology distal to the nerve graft demonstrated increased axon counts and a shift to larger fiber diameters due to GDNF MS treatment. The sustained delivery of GDNF to the implanted ANA achieved in this study demonstrates the promise of this DDS for the management of severe nerve injuries in which allografts are placed.

Statement of Significance

This work addresses the common clinical situation in which a nerve gap is bridged using acellular nerve allografts. However, these allografts are not as effective in supporting nerve regeneration as the gold standard method of autografting. The novel local drug delivery system used in this study provides sustained and controlled release of glial cell line-derived neurotrophic factor (GDNF), one of the most potent neurotrophic factors, which significantly improves nerve regeneration following severe nerve injuries. Results from this research will provide a mean of improving nerve allografts with locally delivered GDNF. This strategy may lead to a novel "off the shelf" alternative to the current management of severe nerve injuries.

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1. Introduction

Despite substantial improvements in microsurgical techniques, patients with peripheral nerve injuries rarely recover fully [1,2]. Direct end-to-end repair of the transected peripheral nerve supports limited recovery following injury [3–5]. However, in many clinical situations, there is not enough nerve tissue to allow a tension free reconstruction [6,7]. In these cases, the current surgical standard consists of using an autograft, in which a nerve graft from the same patient is used to bridge the nerve gap. Although autografts provide tension-free repair, they require a second operative site which necessitates additional operative time, a permanent scar, donor sensory loss, and could result in persistent

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postoperative pain [8]. Moreover, due to the limitation in the available length, nerve autografts may not be feasible in cases where extensive reconstruction is required [8].

An alternative to autografting is the use of processed nerve allografts, or acellular nerve allografts (ANA)s [9]. ANAs retain the scaffold of nerve tissue but are made to be non-immunogenic to the recipient by a variety of processing methods, such as repeated freeze–thaw cycles; cold preservation; and decellularization with detergents [8,10,11]. Thus, ANAs provide a biological substrate for nerve regeneration without the requirement of immunosuppression. However, they have non-therapeutic levels of neurotrophic factors especially compared to normal denervated nerve stumps in which several growth factors are upregulated after injury [12]. Given that regenerating nerve fibers preferentially elongate toward sources of neurotrophic factors [13,14], replenishing the ANAs with key neurotrophic factors should enhance nerve gap regeneration. ANAs have been used clinically in patients for several years [15], and we questioned whether the ability of these allografts to support nerve regeneration could be improved by supplementation with key neurotrophic factors lacking in the commercially available ANA.

Delivery of neurotrophic factors holds promise in enhancing outcome following nerve injury [16]. Neurotrophic factors, such as brain-derived neurotrophic factor, nerve growth factor, and glial cell line-derived neurotrophic factor (GDNF), which are essential for peripheral nervous system development, have been shown to promote axon regeneration and enhance functional recovery [12,17,18]. However, the challenge for achieving a clinically suitable application for GDNF is its localized and sustained release to the nerve injury site [12,18]. Current investigational methods of GDNF local delivery include viral transfected Schwann cells [19,20], and catheter/mini-osmotic pump systems [21]. While viral transduction of primary cells generates local release, regulation of GDNF release is difficult to manage and can result in excess and toxic GDNF release. In addition, clinical translation may be a significant regulatory challenge and these methods are not currently approved for clinical use. Osmotic pump delivery systems, despite providing sustained and localized release, can hinder recovery due to risk of infection and even nerve compression secondary to capsular fibrosis [3]. A sustained and tunable delivery from a biodegradable and biocompatible system is therefore preferred to effectively delivery GDNF to the injured nerve.

Previously, we developed a microsphere-based biodegradable drug delivery system (DDS) supporting sustained release to the injured nerve over periods of days to weeks [22,23]. This DDS, consisting of fibrin gel containing GDNF microspheres, significantly improves axon regeneration and functional recovery after delayed nerve repair [22,23]. In this study, we combined the DDS composite system with the rat analogue of the clinically-used nerve allograft to determine the extent to which this new hybrid DDS-ANA biomaterial supported nerve regeneration.

2. Materials and methods

All chemicals were obtained from Sigma-Aldrich (St. Louis, MO, USA) unless otherwise specified.

2.1. GDNF encapsulation in PLGA microsphere

Glial cell line-derived neurotrophic factor (GDNF) was encapsulated in poly(lactic-co-glycolic acid) (PLGA) microsphere (MS) using a water/oil/water double emulsion, solvent evaporation method. Briefly, an inner aqueous solution of 100 μL consisting of 250 μg GDNF (Peprotech, Rocky Hill, NJ) and 12.5 mg heparin was mixed with 230 mg PLGA 50/50 (Wako, Japan and Lactel Absorbable Polymers, Cupertino, CA, Table 1) and 12.5 mg $MgCO_3$ in 1 mL dichloromethane (DCM)/acetone (75%/25%). The mixture was sonicated for 45 s using a 3 mm probe sonicator (Vibra-Cell™ VCX 130; Sonics and Materials, CT, USA) at 30% power. The resulting emulsion was added to 25 mL of 2.5% aqueous poly(vinyl alcohol) (PVA) solution containing 10% NaCl and homogenized at 6000 rpm for 60 s. The secondary emulsion was then added to 250 mL aqueous solution of 2.5% PVA and 10% NaCl. The mixture was stirred for 3 h with venting to allow the hardening of the microspheres by complete evaporation of the organic solvent. The hardened microspheres were collected and washed by centrifugation, lyophilized, and stored at −20 °C until use.

2.2. GDNF microsphere characterization

Microsphere mean diameter and size distribution were measured via static light scattering using a Malvern Mastersizer 2000 laser diffraction particle sizer (Malvern Instruments Ltd, UK), using refractive indices of 1.33 and 1.59 for water and PLGA, respectively. Encapsulation efficiency was measured by dissolving an appropriate mass of microspheres in 1 mL dimethyl sulfoxide (DMSO) for 1 h at 37 °C followed by addition of 10 mL of 0.05 M NaOH with 0.5% w/v sodium dodecyl sulfate (SDS) and further shaking for 1 h at room temperature. The amount of GDNF was quantified by an enzyme-linked immunosorption assay (ELISA) for human GDNF according to the manufacturer's instructions (R&D Systems, Minneapolis, MN). The absorbance was read at 450 nm with an optical subtraction at 540 nm using a multi-well plate spectrophotometer, and sample concentrations were calculated from a standard curve of known GDNF concentrations. Drug loading was determined as the GDNF mass per mg of microspheres; encapsulation efficiency was the measured drug loading of the microspheres divided by the theoretical maximum drug loading.

2.3. GDNF DDS composite construction and in vitro release

Fibrin gel (80 μL total volume) was constructed by mixing equal parts fibrinogen (75–115 mg/mL, 40 μL) and thrombin (5 IU/mL, 40 μL) obtained from a Tisseel® glue kit (Baxter Healthcare, IL, USA), and then re-suspended according to the manufacturer's instructions. Fibrin gels were loaded with microspheres by incorporating 5 mg of microspheres into the thrombin solution before it was mixed with fibrinogen to form a gel. *In vitro* release of GDNF from fibrin loaded with microspheres was assessed by using 80 μL gels in 2 mL siliconized centrifuge tubes (Fisher Scientific). The time course of release was measured by incubating the fibrin gels in 1 mL of phosphate buffered saline (PBS) containing 1% bovine serum albumin (BSA) at 37 °C under constant gentle agitation by vortex. The PBS was collected and ELISA assays were performed

Table 1
PLGA description used in the drug delivery system synthesis.

Formulation name	PLGA inherent viscosities (dL/g)	PLGA average molecular weight (Da)	GDNF initial loading (%wt/wt in microspheres)	Encapsulation efficiency (%)
2-week release formulation	0.088–0.102	5000	250 mg (0.05%)	78 ± 3
4-week release formulation	0.15–0.25	6700	250 mg (0.05%)	78 ± 3

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