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Affinity-based release of glial-derived neurotrophic factor from fibrin matrices enhances sciatic nerve regeneration ☆

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Abstract

Glial-derived neurotrophic factor (GDNF) promotes both sensory and motor neuron survival. The delivery of GDNF to the peripheral nervous system has been shown to enhance regeneration following injury. In this study we evaluated the effect of affinity-based delivery of GDNF from a fibrin matrix in a nerve guidance conduit on nerve regeneration in a 13 mm rat sciatic nerve defect. Seven experimental groups were evaluated which received GDNF or nerve growth factor (NGF) with the delivery system within the conduit, control groups excluding one or more components of the delivery system, and nerve isografts. Nerves were harvested 6 weeks after treatment for analysis by histomorphometry and electron microscopy. The use of the delivery system (DS) with either GDNF or NGF resulted in a higher frequency of nerve regeneration vs. control groups, as evidenced by a neural structure spanning the 13 mm gap. The GDNF DS and NGF DS groups were also similar to the nerve isograft group in measures of nerve fiber density, percent neural tissue and myelinated area measurements, but not in terms of total fiber counts. In addition, both groups contained a significantly greater percentage of larger diameter fibers, with GDNF DS having the largest in comparison to all groups, suggesting more mature neural content. The delivery of GDNF via the affinity-based delivery system can enhance peripheral nerve regeneration through a silicone conduit across a critical nerve gap and offers insight into potential future alternatives to the treatment of peripheral nerve injuries.

Keywords: Drug delivery; Growth factor; Nerve guidance conduit; Peripheral nerve graft; Tissue engineering

1. Introduction

Despite recent advances in the understanding of peripheral nerve injury and regeneration, functional outcomes are still suboptimal. In nerve transection injuries, the current standard of care is a primary end-to-end repair. In nerve gap injuries, when tension precludes a primary repair, an autograft is used to provide a scaffold for the regenerating nerve. This procedure, however, has limitations due to donor site availability and morbidity [1,2]. One alternative to autografting is the use of a nerve guidance conduit (NGC). NGCs facilitate bridging the gap between a proximal and a distal nerve, protect regenerating axons from infiltrating scar tissue, and allow the microenvironment of the regenerating nerve to be manipulated by controlling biochemical and physical contents [1,3].

A variety of materials have been investigated for use as scaffolds to fill the lumen of a NGC, including the extracellular matrix proteins collagen [4,5], fibronectin [6] and laminin [5], as well as naturally derived matrices such as agarose [7,8] and alginate [9,10]. Fibrin has also been used

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as a biomaterial scaffold to support neural regeneration within an NGC [11–13] and may offer an advantage over other materials because it naturally forms within an empty silicone conduit connecting the damaged ends of rat sciatic nerve [14]. Furthermore, fibrin contains sites for cell binding via integrin receptors [15], including cell binding sites for Schwann cells [16], which may facilitate cellular migration.

Numerous drug delivery methods have also been used with NGCs [17–22]. However, diffusion-based release of growth factors from degradable polymers is the most common delivery method [17,18,23]. One shortcoming of this approach is that the release rate cannot be modulated or controlled by cells during regeneration. One alternative is to use an affinity-based delivery system (DS) that allows the release of growth factors to be controlled by cell-based degradation of the delivery system [24]. Our laboratory has developed an affinity-based delivery system that sequesters heparin-binding proteins within a fibrin matrix using non-covalent interactions [25,26]. This system contains a bi-domain peptide containing a transglutaminase substrate domain and a heparin-binding domain. Based on the α_2-plasmin inhibitor substrate [27,28], the peptide is able to cross-link into the fibrin matrix during polymerization via the transglutaminase activity of Factor XIIIa, leaving the other domain free to interact [25,26]. This heparin-binding domain has the capability to sequester various neurotrophic factors due to their ability to bind to heparin via the sulfated domains on the heparin [29]. This delivery system has been used with a variety of growth factors in many potential treatment applications [11,24,30–33]. Specifically, we have characterized the effect of affinity-based delivery of nerve growth factor (NGF) on peripheral nerve regeneration [11].

Glial-derived neurotrophic factor (GDNF) has shown promise in the treatment of peripheral nerve injuries. While GDNF has been found to promote the survival of both sensory and motor neurons, multiple studies report it to be the most potent motor neuron trophic and survival factor [34–39]. GDNF expression in peripheral nerves is also up-regulated significantly in the distal stump of injured sciatic nerve, as well as in the corresponding muscle [40,41]. Given the ability of GDNF to enhance peripheral nerve regeneration [17,18,20], we chose to examine controlled delivery of GDNF from our affinity-based delivery system in vitro and found that GDNF could be retained and released from the delivery system in a biologically active form [42].

In the present study, we evaluated the effects of controlled release of GDNF from a fibrin matrix containing our affinity-based delivery system within a NGC on nerve regeneration in vivo using a rat sciatic nerve injury model. We included NGF in the current study for comparison to our previous study. We hypothesized that controlled delivery of GDNF would enhance nerve regeneration and have histomorphometric equivalence to a nerve isograft.

2. Materials and methods

2.1. Experimental animals

Adult male Lewis rats (Harlan Sprague–Dawley, Indianapolis, IN), each weighing 250–300 g, were used in this study. All surgical procedures and peri-operative care measures were performed in strict accordance with the National Institutes of Health guidelines and were approved by the Washington University Animal Studies Committee. All animals were housed in a central animal facility, given a rodent diet (PicoLab Rodent Diet 20 #5053, PMI Nutrition International) and water ad libitum. After surgical procedures, animals recovered in a warm environment and were closely monitored for 2 h. Animals were then returned to the animal facility and monitored for weight loss, infection and other morbidities.

2.2. Experimental design

Eighty-four animals were randomized into seven groups ($n = 12$), as shown in Table 1. An additional six animals served as sciatic nerve isograft donors. In all experimental groups, the sciatic nerve was transected and a 5 mm segment was excised just proximal to the trifurcation of the nerve. The nerve was repaired with a 15 mm silicone conduit containing fibrin matrices with or without the delivery system and growth factor. One millimeter of nerve was incorporated into each end of the conduit to create a 13 mm nerve gap, exceeding the "critical gap" of spontaneous rat sciatic regeneration through silicone conduits by 3 mm [14,43] (Fig. 1). Group I served as the untreated control group and received an empty conduit. Groups II, III and IV were additional control groups, receiving conduits containing fibrin alone, fibrin with the delivery system (no growth factor) or fibrin with the growth factor but no delivery system, respectively. These groups examined the isolated effects of the delivery system components. The remaining groups (V, VI) were implanted with conduits containing the fibrin matrix containing the delivery system with doses of GDNF or NGF, which were selected based upon in vitro DRG dose studies [42] and preliminary data obtained from dose–response pilot studies in the sciatic nerve model (test doses included 25, 50, 100, and 250 ng ml^{-1} GDNF with the delivery system). Group VII served as a positive control receiving reversed nerve isografts from syngeneic donor animals.

2.3. Preparation of fibrin matrices

Fibrinogen solutions were prepared by dissolving human plasminogen-free fibrinogen in deionized water at 8 mg ml^{-1} for 1 h and dialyzing vs. 4 l of Tris-buffered saline (TBS) (33 mm Tris, 8 g l^{-1} NaCl, 0.2 g l^{-1} KCl) at pH 7.4 overnight to exchange salts present in the protein solution. The resulting solution was sterilized by filtration through 5.0 and 0.22 μm syringe filters, and the final fibrinogen con-

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