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Diffusion of soluble factors through degradable polymer nerve guides: Controlling manufacturing parameters

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Abstract

Nerve guides are cylindrical conduits of either biologically based or synthetic materials that are used to bridge nerve defects. While it is well known that a critical aspect of nerve regeneration is the delivery of oxygen and nutrients to the surviving nerve tissue, several guide parameters that determine the permeability of nerve guides to nutrients are often overlooked. We have reproducibly manufactured poly(caprolactone) (PCL) nerve guides of tailored porosity percentage, wall thickness and pore diameter through a dip-coating/salt-leaching technique. In this study, these three parameters were varied to measure the response of glucose and lysozyme diffusion through the guide wall. In addition, nerve guide permeability following protein fouling studies was examined. Based on the results from this study, it was determined that at high porosity percentages (80%), decreasing the pore diameter (10–38 μm) was a measurable method of decreasing the lysozyme permeability of PCL nerve guides while not creating a loss of glucose permeability. PCL fouling studies were used to fine-tune the desirable nerve guide wall thickness. Results indicated that nerve guides 0.6 mm thick decreased the loss of lysozyme to almost 10% without significantly diminishing glucose (nutrient) permeability. These results will be utilized to optimize nerve guide parameters for future in vivo applications.

Keywords: Guided tissue regeneration; Polymer processing; Nerve regeneration; Poly(caprolactone); Nerve guide permeability

1. Introduction

When a peripheral nerve undergoes complete neurotmesis during injury, where both the nerve and nerve sheath are disrupted, end-to-end coaptation of the severed nerve stumps is the preferred surgical therapy. However, when nerve ends cannot be directly reconnected without tension, a bridging device must be implanted between the proximal and distal nerve stump. Although there are some disadvantages to the use of nerve autograft, it is the preferred clinical choice for use as a tissue-based nerve-bridging device. However, for patients who lack an adequate amount of extraneous tissue for use as nerve graft material or in surgical environments where second harvesting procedures for graft material is not ideal (such as in combat casualty care), the use of synthetic nerve conduits to bridge nerve gaps is necessary.

The requirements of synthetic nerve guides are to mechanically support and direct axonal sprouting between injured nerve stumps, prevent fibrous tissue ingrowth into the injury site and retain secreted neurotrophic factors secreted by the damaged nerve ends [1]. Nerve guides have been constructed from a variety of both biological and synthetically based materials [2] and with a variety of design additives such as luminal fillers [3] or delivered growth factors [4]. Within the enormous breadth of nerve guide literature, many of the mechanical requirements of implantable nerve guides have been defined. For example, the guide must be of the proper dimensions: the length must be long enough to bridge the gap without tension and the width must house the nerve stumps without compression. Fur-

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thermore, the material must be mechanically sound to resist tearing from sutures, yet soft enough to avoid tissue inflammation. Finally, the nerve guide material must be slowly degrading so as to provide support for the entire regeneration period and have a low degree of swelling to avoid compression of the nerve injury. However, other design aspects are less clear.

It was initially assumed beneficial to place transected nerve stumps in nonporous silicone tubes because the conduit conveyed fluid and cells from the regenerating nerve stumps to the injury site. However, a 1985 study by Jenq and Coggeshall showed that by allowing the extracellular fluid and its cells into the lumen of the guide through macroscopic holes in the guide wall, the number of both myelinated and unmyelinated axons was significantly increased [5]. Following this publication, additional work has confirmed that a critical facet in nerve regeneration is the delivery of oxygen and nutrients to the surviving nerve tissue [6–9]. Presumably, nerve guides should be developed to maximize the influx of oxygen and nutrients from interstitial fluid through pores in the guide wall. However, wall porosity is restricted because of the opposing needs to prevent inflammatory cells from migrating into the lumen, and to minimize the diffusion of growth factors out of the guide lumen.

The purpose of this study was to optimize nerve guide fabrication parameters such that lysozyme diffusion through poly(caprolactone) (PCL) conduit walls was minimized while nutrient (glucose) permeability was maximized. To achieve this, the effect of three fabrication variables (wall thickness, pore size and porosity percentage) on both glucose and protein diffusion was tested. While it is unlikely that protein loss from a porous scaffold will be entirely eliminated, the authors determined that a 10% loss of protein payload would be acceptable. However, because Schwann cell survival has been indicated as being limited by blood nutrient diffusion, we also aimed to determine the maximum nerve guide permeability to glucose.

2. Materials and methods

2.1. Reagents

All chemicals were analytical grade or purer and were purchased from commercial suppliers. PCL (average Mw ~65,000, average Mn ~42,500, pellets), ethyl acetate, ninhydrin, sodium chloride (crystalline, fine), poly(vinyl alcohol) (PVA, average Mw 9000–10,000, 80% hydrolyzed), poly(DL-lactide-co-glycolide) (lactide:glycolide (50:50), Mw 40,000–75,000 U), lysozyme from chicken egg white, glucose assay kit (GAGO-20), tetrahydrofuran, 1,6-hexanediamine and D-(+)-glucose were all purchased from Sigma–Aldrich (St Louis, MO). Glass capillary tubes, premium glass microscope slides and Tissue-Tek Cryo-OCT Compound were purchased from Thermo Fisher Scientific (Waltham, MA). The Micro Bicinchoninic Acid (BCA) Protein Assay Kit (23235) was purchased from Pierce (Rockford, IL). 26G Needles were purchased from Becton Dickinson (Franklin lakes, NJ). Poly(L-lactide) was purchased from Durect Corporation (Pelham, AL).

2.2. Polymer nerve guide fabrication

Permeable nerve guides were created by dissolving PCL in ethyl acetate. To the dissolved polymer solution, sodium chloride impregnation was accomplished by adding NaCl in a specified v/v ratio (NaCl:PCL). Large crystals of NaCl were first ground into a fine powder with a mortar and pestle and then sifted through sieves of known mesh diameter. Glass capillary mandrels 1.5 mm in diameter were coated with a 17% w/v aqueous solution of PVA, air dried and then immersed into the polymer slurry creating NaCl/PCL mandrel coatings. The ethyl acetate was allowed to evaporate for a minimum of 10 min between successive mandrel immersions into the polymer slurry. After the completion of the dip-coating process, the resulting polymer conduits were submerged in distilled water to allow for salt and PVA dissolution, and the glass mandrels were removed. Porosity percentage was varied by altering volume percentages of sodium chloride in the polymer–solvent slurry. The nerve guide wall thickness was adjusted by varying the number of immersions performed in the dip-coating technique used to create the guides.

2.3. Nerve guide porosity measurement

Wall porosity percentage was determined by scanning electron microscopy (SEM) image analysis. PCL nerve guides were dried, cut into thin sections with a razor blade and mounted on metal stubs covered in copper double-sided tape. The guides were then gold-coated to 3.5 nm thick using a Cressington 108 auto sputter-coater (Cressington, Watford, UK), and viewed with a JEM-6330f (JEOL, Peabody, Ma) scanning electron microscope operated at 5 kV acceleration. Low-magnification images were used for image analysis as previous literature has shown good agreement of image analysis of material porosity with actual porosity values (Fig. 1A) [10]. To determine the guide porosity, the proportion of pixels dedicated to "wall area" vs. "pore area" was determined with ImageJ software [11]. Because of the subjective nature of thresholding grayscale images, Otsu's method of thresholding was employed to decipher grayscale levels within the image that were considered polymer or empty space, creating a binary image from which pixel areas could be calculated (Fig. 1B) [10]. For bi-level thresholding, Otsu's method optimizes the threshold such that the variance between light and dark pixels is maximized [12] and, as a result, the variance in pixels associated with the pores and walls is minimized.

2.4. Nerve guide wall thickness measurements

To determine the wall thickness of PCL nerve guides, five batches of nerve guides were fabricated using the dip-

ID	Title	Pages
1936	Diffusion of soluble factors through degradable polymer nerve guides: Controlling manufacturing parameters	11