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Beagle sciatic nerve regeneration across a 30 mm defect bridged by chitosan/PGA artificial nerve grafts

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ABSTRACT

Longitudinally oriented microstructures are essential for a nerve scaffold to promote the significant regeneration of injured peripheral axons across nerve gaps. In the current study, we present a novel nerve-guiding collagen-chitosan (CCH) scaffold that facilitated the repair of 30 mm-long sciatic nerve defects in beagles. The CCH scaffolds were observed with a scanning electron microscope. Eighteen beagles were equally divided into CCH group, autograft group and non-graft group. The posture and gait of each dog was recorded at 12 and 24 weeks after surgery. Electrophysiological tests, Fluoro-Gold retrograde tracing test, Histological assessment of gastrocnemius and immunofluorescent staining of nerve regeneration were performed. Our investigation of regenerated sciatic nerves indicated that a CCH scaffold strongly supported directed axon regeneration in a manner similar to that achieved by autologous nerve transplantation. In vivo animal experiments showed that the CCH scaffold achieved nerve regeneration and functional recovery equivalent to that achieved by an autograft but without requiring the exogenous delivery of regenerative agents or cell transplantation. We conclude that CCH nerve guides show great promise as a method for repairing peripheral nerve defects.

Introduction

Peripheral nerve regeneration and functional recovery are current clinical challenges. For short nerve injuries, direct end-to-end nerve sutures are proposed as standard treatment. The standard technique to treat long-lesion gaps involves transplanting autologous nerve grafts (autografts) from uninjured sites to the injured site to form a bridge between the two nerve stumps [1,2].

However, the use of autografts has several disadvantages and limitations: secondary surgical injury to the donor site, limited availability of donor nerves, permanent loss of donor nerve function and mismatches in tissue structure and size. Therefore, it is imperative to develop alternatives to conventional nerve autografts [3].

Bridging long nerve gaps with tissue-engineered grafts has produced promising results and has received extensive attention. Tissue-engineered grafts are generally developed by combining biomaterial scaffolds, seed cells, and biologically active molecules. The inner microstructural properties of the scaffold are the predominant factors that determine the efficacy of a tissue-engineered graft in bridging nerve gap lesions [4,5]. Our recent studies show that several scaffolds with oriented structures, such as fibres and grooves, are capable of physically guiding the linear growth of regenerated axons to some extent. Since the basal lamina microchannels in normal nerves are known to play a significant role in guiding the linear growth of regenerated axons, scaffolds with dimensions resembling basal lamina microchannels are expected to provide a promising alternative for bridging nerve gaps [6–8].

In the present study, we used a novel, longitudinally oriented, nerve-guiding scaffold with inner dimensions resembling the basal lamina microchannels of normal nerves, and we evaluate its efficacy in bridging 30 mm-long sciatic nerve defects in dogs using a combination of morphological and functional techniques. We found that this (CCH) scaffolds can effectively promote the nerve regeneration and functional recovery after injuries. All these finding highlight the CCH in promoting peripheral nerve regeneration and functional recovery after nerve injury in clinical research.

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Materials and methods

Fabrication of collagen-chitosan scaffolds

Briefly, collagen-chitosan (CCH) scaffolds with longitudinally oriented microchannels were prepared using a "unidirectional freezing" method followed by freeze-drying [4]. The CCH suspension was produced by mixing type I collagen (2.8 wt%; Sigma, St. Louis, MO) and chitosan (0.7 wt%; Sigma) in a solution of 0.05 M acetic acid (pH 3.2) using an overhead blender (IKA Works, Wilmington, NC). The temperature of the suspension was maintained at 4–8 °C during the mixing process to avoid collagen fibre denaturation. The scaffolds were sterilized via exposure to 20 kGy ^{60}Co radiation before the animal study.

Microstructure observation

The cross-linked CCH scaffolds were sectioned in longitudinal and transverse planes and viewed with a scanning electron microscope (SEM; S-3400N; HITACHI, Tokyo, Japan) at an accelerating voltage of 5 kV. Before observation, the samples were dehydrated using a series of increasing concentrations of ethanol followed by brief vacuum drying. The dry scaffolds were sputter-coated with gold at 40 mA and then observed with a SEM.

Animals and surgical procedure

In this study, eighteen adult male beagle dogs weighing 10–12 kg were obtained from the "DiLePu" Biological Resources Development Company (Xi'an, Shaanxi Province, China). All the animal tests were carried out in accordance with the US National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals published by the US National Academy of Sciences (http://oacu.od.nih.gov/regs/index.htm) and were approved by the Administration Committee of Experimental Animals, Shaanxi Province, China. The dogs were randomly divided into three groups: (i) six in a CCH graft group; (ii) six in an autograft group as the positive control; and (iii) six in a non-graft group as the negative control.

Each animal was anaesthetized before surgery by an intraperitoneal injection of sodium pentobarbital (30 mg/kg body weight) into its sciatic nerve. The sciatic nerve was exposed after making a skin incision and splitting the underlying muscles in the left lateral thigh. A 26 mm-long segment of sciatic nerve was then resected, leaving a 30 mm-long defect (longer because of the retraction of the nerve ends). In the CCH group, the nerve defects were bridged with CCH scaffolds. The CCH scaffolds and the silicone conduits were sutured to both the proximal and distal nerve stumps with 10/0 nylon sutures of monofilament polyamide under 40× magnification (Fig. 1). In the autograft group, the removed nerve segments were rotated 180° and re-implanted with epineural sutures. In the non-graft group, the nerve gaps were left unbridged. In all animals, the skin was then closed with 3–0 stitches and liquid topical skin adhesive. After surgery, the animals were returned to their cages and allowed to recover for 12 and 24 weeks.

General observation

After surgery, all dogs were housed, routinely fed, and monitored for changes in their appearance, appetite, response and locomotion. The posture and gait of each dog were video recorded and documented at 12 and 24 weeks after surgery.

Electrophysiological assessment

Twenty-four weeks after surgery, electrophysiological tests were performed on all animals. A bipolar stimulating electrode was placed under the sciatic nerve at a location 10 mm proximal to the graft site. A recording electrode was placed in the gastrocnemius muscle, and compound muscle action potentials (CMAPs) were recorded. Normal CMAPs were measured on the contralateral unoperated side. The CMAP peak amplitude, CMAP latency of onset, and nerve conduction velocity (NCV) values were calculated [9].

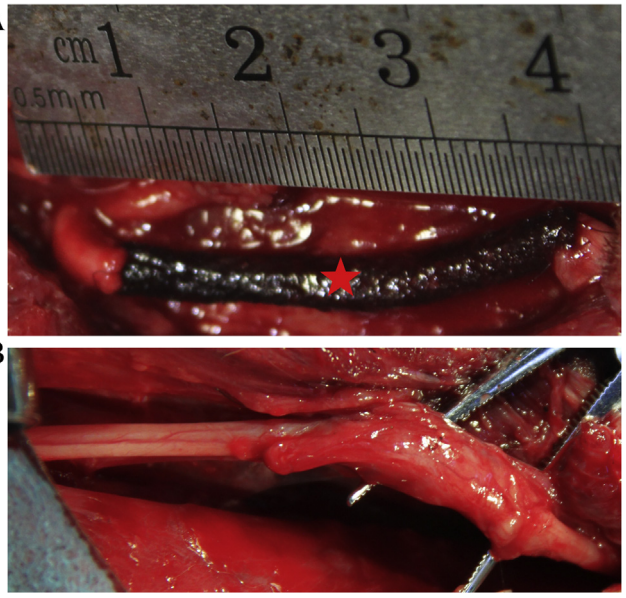

Fig. 1. Gross view obtained immediately after bridge implantation of the CCH scaffolds (asterisk) into a 30 mm-long sciatic nerve defect in dogs (A). The CCH graft groups at 24 weeks post-operative (B). Minimal scale = 1 mm.

Fluoro-Gold retrograde tracing

At 24 weeks after surgery, retrograde labelling was performed, and back-labelled cells were counted. After the sciatic nerve was exposed, 5 mL of 4% Fluoro-Gold (FG) (Biotium, Hayward, CA) solution was intraneurally injected into the sciatic nerve trunk of each dog at a point 10 mm distal to the graft. The incision was then sutured, and the dog was returned to its cage for a period of 7 days to allow the retrograde tracers to travel to the neuronal cell bodies. Thereafter, the dogs were intracardially perfused with 4% (w/v) paraformaldehyde in 0.1 M phosphate buffer. The vertebral canal was opened, and the lumbar spinal cord was exposed. Then, L4, L5, and L6 and the dorsal root ganglia (DRG) were harvested. The procured tissues were post-fixed in buffered 4% paraformaldehyde for 4 h and then cryoprotected in 30% sucrose overnight at 48 °C. Transverse sections (thickness, 25 μm) were prepared from the spinal cords, and longitudinal sections (thickness, 16 μm) were prepared from the dissected DRG. All of the sections were mounted on poly-lysine pre-coated glass slides and then viewed and imaged under a fluorescence microscope (BX-60; Olympus). The number of FG-labelled spinal cord motoneurons and the number of FG-labelled DRG sensory neurons were directly counted [10,11].

Histological assessment

The gastrocnemius muscles were harvested from both sides of each deeply anaesthetized animal, and their wet weights were measured [12]. To analyse nerve specimens, transverse sections were obtained at four sites for each specimen (i.e., the proximal sciatic (PN), proximal graft (PG), distal graft (DG) and distal sciatic nerves (DN)).

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