Brain-Derived Neurotrophic Factor–Enriched Collagen Tubule as a Substitute for Autologous Nerve Grafts

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Background: Autologous nerve interposition grafts are frequently harvested by head and neck surgeons. The sacrifice of these donor nerves guarantees some degree of morbidity, including sensory loss, additional incision sites with associated potential complications, and prolonged operative time. An alternative to autologous nerve grafting is, therefore, desirable.

Objective: To determine if a collagen tubule (CT) filled with either a plain collagen gel or a brain-derived neurotrophic factor (BDNF)–enriched collagen gel could be used to achieve functional and histologic outcomes equivalent to an autologous nerve graft in bridging a 15-mm nerve gap in the rabbit facial nerve.

Design: A prospective, randomized, blinded animal study with a control group.

Methods: Thirty rabbit facial nerves were resected (15-mm segments) to create nerve gaps. The gaps were bridged using 1 of 3 methods, assigned randomly: a reversed facial nerve (control), a collagen gel–filled CT, or a BDNF-enriched collagen gel–filled CT. The animals were evaluated after 6 weeks in a blinded fashion for functional nerve recovery, axon count, and axonal diameter.

Results: There were no significant differences between the autologous nerve graft group, the collagen gel–filled CT group, or the BDNF-enriched collagen gel–filled CT group (n=10 for each group) for functional nerve recovery (P=.94). The mean axon count and the mean axonal diameter were highest in the BDNF-enriched collagen gel–filled CT group, but these differences failed to reach statistical significance (P=.18 and .96, respectively).

Conclusions: Collagen tubules filled with BDNF-enriched collagen gel appear to be at least as good as autologous nerve grafts for bridging short facial nerve gaps. Larger experimental studies are warranted to determine if clinical trials are justified.


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UTOLOGOUS NERVE interposition grafts are frequently harvested by head and neck surgeons. The choices for donor nerve include the sural nerve, the greater auricular nerve, and the medial antebrachial cutaneous nerve, among others. The sacrifice of these nerves guarantees some degree of morbidity, including sensory loss, additional incision sites with associated potential complications, and prolonged operative time. Furthermore, autologous nerves may have significant limitations, including caliber mismatch, inadequate length, and susceptibility to crush injury during harvest. An alternative to autologous nerve grafting is, therefore, desirable.

Bioengineered artificial conduits have shown promise as substitutes for grafts to bridge short nerve gaps. Outcomes have been enhanced by filling the conduits with various extracellular matrix components or growth factors, which help to sustain the growing nerve. Collagen matrix has been demonstrated to support neural growth in silicone tubule and collagen tubule (CT) models. Similarly, brain-derived neurotrophic factor (BDNF) has shown promise when applied after neurorrhaphy and when used to sustain growth across nerve gaps.

The facial nerve is an important model for nerve grafting because it is sometimes necessary to sacrifice the nerve for oncologic reasons, and because of clinical limitations in available length. Anatomic constraints make it difficult to mobilize the human facial nerve to overcome more than a 15-mm defect; larger gaps usually require a nerve graft. Therefore, an artificial conduit capable of bridging a 15-mm gap in a facial nerve could have immediate clinical applicability.

We sought to determine if a CT filled with either a plain collagen gel or a...
MATERIALS AND METHODS

ANIMALS

The left and right facial nerves of 13 male New Zealand white rabbits weighing 2.6 to 3.0 kg were used for the investigation, yielding a total of 30 nerves. After a 15-mm segment of the buccal branch of the facial nerve was resected, nerve reconstruction was undertaken using 1 of 3 methods, assigned randomly: a reversed facial nerve (control), a collagen gel–filled CT (CT-gel), or a BDNF–enriched collagen gel–filled CT (CT-BDNF). Animals were observed daily to inspect the surgical incisions and to ensure proper wound healing. The protocol was approved by the Stanford University Administrative Panel on Laboratory Animal Care.

TUBULES

The tubules were synthesized from highly purified type I collagen (<0.2% hexosamine and <0.1% trichloroacetic acid–insoluble residues, with ≤4 tyrosine residues per molecule), derived from bovine deep flexor tendon (Integra LifeSciences, Plainsboro, NJ). The fibrillar structure of the collagen was maintained throughout processing. The structural stability of the tubules was increased by gaseous formaldehyde cross-linking, which also controls the rate of in vivo resorption. Previous studies have shown that the tubules are freely permeable to macromolecules as large as bovine serum albumin (molecular weight, 68 kd).

The tubules were cut to 20-mm lengths, each with an inner diameter of 2.5 mm. They were initially hydrated in lactated Ringer solution and then filled with plain collagen gel or BDNF–enriched collagen gel. The collagen gel used was composed of purified collagen dissolved in 0.012 N hydrochloric acid for a final concentration of 3 mg/mL (Cell Prime; Collagen Biomaterials, Palo Alto, Calif). The BDNF was provided by Regeneron Pharmaceuticals, Tarrytown, NJ, and used at a final concentration of 300 µg/mL.

SURGICAL TECHNIQUE

The anesthesia consisted of a subcutaneous injection of atropine, 0.05 mg/kg, 15 minutes before the procedure, followed by an intramuscular injection of ketamine hydrochloride, 35 mg/kg, and xylazine hydrochloride, 5 mg/kg. The anesthetic depth was monitored by toe pinch and adjusted as needed. The respiratory and heart rates were monitored every 15 minutes according to Stanford University institutional guidelines.

Each rabbit underwent bilateral exposure and resection of a 15-mm segment of the buccal branch of the facial nerve. The nerves were then reconstructed, according to randomization, with 1 of the following: a reversed segment of the facial nerve, CT-gel, or CT-BDNF. A single 10-0 monofilament nylon suture (Ethicon, Inc, Somerville, NJ) was used to secure each end of the tubule, and 4 to 5 epineurial sutures were used on each end of the nerve graft. The surgeon was blinded to the type of tubule used. The wounds of the animals in each of the 3 groups were closed with absorbable sutures.

Postoperative analgesia consisted of subcutaneous injections of buprenorphine hydrochloride, 0.01 to 0.05 mg/kg, every 6 to 12 hours as needed. The animals were monitored postoperatively for increased heart or respiratory rate as an indicator of distress. Six weeks after nerve reconstruction, the animals were killed by a lethal injection of pentobarbital sodium.

FUNCTIONAL ANALYSIS

Six weeks after surgery, functional assessment of the animals was undertaken, as previously described. All rabbits were observed for spontaneous movements of the upper lip and whiskers. Induced movements of the upper lip and whiskers were then elicited by performing a gentle midline nasal tap. Movement was scored on a 5-point scale (0–4). The observer was blinded to the type of nerve reconstruction used.

HISTOLOGIC ANALYSIS

At the time of sacrifice, the nerve graft or tubule was harvested with an additional length of nerve 5 mm proximal and distal to the nerve reconstruction site. The specimen was fixed in buffered 10% formalin solution and then embedded in paraffin and stained with Bielschowsky silver stain.

Morphometric measurements were made of the myelinated axon count and mean axonal diameter for each distal nerve segment using a light microscope (Nikon Alphaphot 2YS2; Technical Instruments, San Francisco, Calif) equipped with a single-chip color video camera (JEDMED CCD model 70-5110; JEDMED Instrument Company, St Louis, Mo) projected onto a color monitor (NEC model PM 1971A; Tokyo, Japan). The monitor was connected to a computer (Performa 6115 CD MacIntosh; Apple, Cupertino, Calif) equipped with a Scion Frame Grabber Card and Scion Image program (model LG3; Scion Corp, Bethesda, Md), which captures images from the video screen and digitizes the analog signal for editing on the computer.

The nerve cross-sectional area (measured in micrometers squared) was determined from the digitized image of the nerve (original magnification ×40). The number of axons was determined by manually counting the axons in 10 randomly selected areas (1290 µm² each) within each nerve section (original magnification ×400). A mean axon count per random 1290-µm² area was calculated and multiplied by the nerve cross-sectional area and then divided by 1290 µm² to obtain the total number of myelinated axons for that specific nerve segment.

The mean axonal diameters were derived from the digitized images of the nerves at original magnification ×100. Twenty axons were chosen randomly and individually outlined on the computer screen. Using the Scion Image program, the areas of the axons were measured, and the diameters were calculated from the areas, assuming a circular geometric shape. The mean axonal diameter of each nerve section was then calculated. The observer remained blinded during the histologic and anatomic evaluation.

STATISTICAL ANALYSIS

Morphometric variables and functional recovery were calculated for each group. These data were entered into a customized spreadsheet and analyzed. Differences in means between the 3 groups were compared using an analysis of variance. Mean differences between the experimental and control groups were compared using the 2-tailed t test. Data are given as mean ± SD unless otherwise indicated.
BDNF-enriched collagen gel could be used to achieve functional and histologic outcomes equivalent to those obtained with an autologous nerve graft in bridging a 15-mm gap in the rabbit facial nerve. The long-term objective of this investigation is to develop a synthetic substitute for autologous nerve grafts that provides better reliability and availability without donor site morbidity.

RESULTS

There were no surgical complications and no perioperative animal deaths. However, 2 of the nerves failed to demonstrate any axonal growth through the reconstruction (1 was in the CT-gel group and 1 was in the nerve graft group); 28 of 30 nerves, therefore, were included in the statistical analysis. An example of the typical appearance of a nerve that was reconstructed with a CT is provided in Figure 1.

HISTOLOGIC ANALYSIS

The axon count was highest in the CT-BDNF group (7210±7001) compared with the CT-gel (3717±1574) and nerve graft (3978±1873) groups, but this difference failed to reach statistical significance (P=.18) (Figure 2).

The axonal diameter was similar among the 3 groups. It was highest in the CT-BDNF group (2.99±0.45 µm) compared with the CT-gel (2.95±0.36 µm) and nerve graft (2.93±0.35 µm) groups, but these differences were not statistically significant (P=.96) (Figure 3). A representative histologic specimen is shown in Figure 4.

FUNCTIONAL ASSESSMENT

Comparison of the mean facial movements (on the 5-point scale) at the time of nerve harvest for the 3 groups revealed no significant differences (P=.94). The movement score was 3.3±0.5 for the animals whose nerves were bridged with the CT-BDNF, 3.2±0.6 for the animals whose nerves were bridged with the CT-gel, and 3.2±0.6 for the animals whose nerves were bridged with a nerve graft (Figure 5).

An artificial nerve graft is desirable because it would minimize patient morbidity that accompanies autologous donor nerve harvest, including the need for longer anesthesia time, postoperative pain, harvest site scar, and sensory or motor deficit. If an artificial conduit can be achieved that is functionally equivalent to the gold standard (an autologous nerve with epineurial repair), it would significantly improve patient care. The evolution of artificial nerve grafts was predicated on an understanding of the processes of exudation, cell proliferation, and collagen synthesis that occur immediately after nerve transection. During nerve growth across a gap, Schwann cells emerge and grow into the fibrin clot from the proximal and distal nerve ends before the growth of axons. Subsequently, it was demonstrated that local microstruc-
tissues (fibrin strands, cell surfaces, and basal laminae) determine axonal orientation. Since Schwann cells precede and guide the growth of axons, the interaction of the Schwann cells with the local environment was considered critical. Early attempts to cross long gaps with silicone tubules were unsuccessful, likely because Schwann cells were unable to adhere to silicone, so the space within the tube was filled with loose connective tissue or fat. The use of a porous collagen matrix overcomes this obstacle by promoting Schwann cell-substrate interaction along the walls of the tube, while allowing local micromolecules to diffuse in and out of the lumen.

We demonstrated in this prospective, randomized, and blinded study that collagen gel-filled CTs provide a satisfactory substitute to autologous nerve grafts. The animals whose nerves were grafted with CTs with either collagen gel or BDNF-enriched gel achieved functional and histologic recovery at least as good as an autologous nerve graft when reconstructing a rabbit facial nerve over a 15-mm gap. There are several distinct advantages to the use of CTs. A CT is easier to place than a nerve graft and requires only a single suture on each end, compared with 4 to 6 sutures for a nerve graft. Furthermore, placement of the CT requires less manipulation of the nerve repair site, thereby minimizing crush damage to the recipient nerve ends.

There are several reasons why BDNF was chosen as the neurotrophic factor to be added to the CT in one group. Originally described in 1982 by Barde et al, BDNF has been shown to undergo retrograde transport to the cell body of motor neurons from skeletal muscle. Brain-derived neurotrophic factor has subsequently been reported to rescue rat motor neurons from naturally occurring cell death in vivo (in the ovum) and to prevent the death of cultured embryonic rat spinal motor neurons in vitro. In vivo, BDNF promotes survival of the facial and sciatic motor neurons after axotomy in newborn rats and has been identified in sciatic nerve Schwann cells. Utley et al used a peripheral nerve injury model to demonstrate that locally administered BDNF enhances the functional recovery of nerves repaired by collagen tubulization. A combination of BDNF and ciliary neurotrophic factor (a neurocytokine) increased the rate of nerve recovery compared with BDNF alone in a similar experiment. Despite these theoretic advantages, BDNF did not appear to foster regeneration any better than plain collagen gel-filled CTs in the current model.

In conclusion, CTs filled with BDNF-enriched collagen gel appear to be at least as good as autologous nerve grafts for bridging short facial nerve gaps. Larger experimental studies are warranted to determine if clinical trials are justified.

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