

Tissue-Engineered Scaffolds Are Effective Alternatives to Autografts for Bridging Peripheral Nerve Gaps

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ABSTRACT

The use of autografts for “bridging” peripheral nerve gaps is limited by lack of suitable donor nerve grafts. Using a tissue-engineering approach, we have designed a three-dimensional scaffold that presents laminin 1 (LN-1) and nerve growth factor (NGF) *in vivo*. Semipermeable polysulfone tubes were used as carriers to introduce the tissue-engineered scaffolds to a 10-mm sciatic nerve gap in adult rats. Two months after implantation, the gross morphology of the regenerated nerve, the success rate of regeneration, and the total number and density of myelinated axons in the tissue-engineered scaffolds matched that observed in autografts. LN-1- and NGF-containing scaffolds performed comparably to autografts when functional measures that include the relative gastrocnemius muscle weight and the sciatic functional index were quantified. Our results demonstrate that tissue-engineered scaffolds match the performance of autografts in an *in vivo* model of peripheral nerve regeneration, raising the possibility of the scaffolds being used clinically instead of scarce autografts.

INTRODUCTION

THERE ARE CURRENTLY two clinical approaches for the repair of peripheral nerve gaps. When the nerve gap is small, the two injured ends are approximated and sutured. When the gap is long (>4 mm), autografts are commonly used as “bridges.” Here, a segment of nerve obtained from the patient (typically, the sural nerve) is grafted between the proximal and distal stumps of the injured nerve. Autograft use is not ideal because their availability is limited, and multiple grafts are often needed.¹ Therefore, it is important to develop alternative approaches for autografts.

The traditional biomaterials-based approach to bridging peripheral nerve gaps uses tubular conduits, termed “guidance channels.” NeuraGen nerve guide, a semipermeable collagen tube developed by Integra NeuroSciences (Plainsboro, NJ), has been used clinically for repairing peripheral nerve injuries.

To further improve regeneration, guidance channel lu-

mens can be filled with Schwann cells, extracellular matrix molecules, and/or neurotrophic factors. In fact, even in saline-filled channels, Schwann cell migration into the lumen is a critical step for successful regeneration.^{2,3} It has been reported that when Schwann cells were preseeded in nerve guidance channels, the injured peripheral nerves regenerated at a faster rate and over longer distances.^{4,5} However, high concentrations of Schwann cells (60–80 million Schwann cells/mL) are needed for functional efficacy⁶ and an unacceptably long time is required to culture sufficient numbers, allowing the injury to worsen. The success of Schwann cells in peripheral nerve regeneration results in part from their production of cell adhesion molecules and neurotrophic factors that mediate neurite attachment and growth,^{7,8} particularly laminin 1 (LN-1) and nerve growth factor (NGF).⁹ Some studies report that the rate of peripheral nerve regeneration significantly increased in guidance channels filled with LN-1-rich Matrigel.^{10,11} However, others report that Matrigel

actually impeded peripheral nerve regeneration, presumably because it contained significant amounts of inhibitory and potentially inflammatory cytokines.¹²

Exogenous NGF facilitates peripheral nerve regeneration across gaps.¹³ However, as growth factors have short half-lives *in vivo* and are unstable in solution, controlled release systems are needed for their delivery.^{14,15}

Because of the inherent limitations of using autografts or cell transplantation approaches as "bridges," we have used a tissue-engineering approach to design a bridge presenting LN-1 and/or NGF to the nerve gap. A 10-mm rat sciatic nerve injury was bridged by semipermeable polymer guidance channels filled with LN-1- and NGF-presenting hydrogel-based scaffolds, and the effects on regeneration were evaluated by histological and functional analysis 2 months after implantation.

MATERIALS AND METHODS

Design of tissue-engineered scaffolds

Design of the tissue-engineered scaffold for spatially and temporally controlled presentation of LN-1 and NGF, respectively. We have designed a two-component system for presentation of extracellular matrix (ECM) and/or trophic factors three-dimensionally in bridge materials *in vivo*. ECM protein LN-1 was presented in the scaffolds three-dimensionally in a homogeneous manner. Neurotrophic factor NGF was loaded in a slow release system and then embedded in the bridge materials.

ECM protein LN-1 was covalently coupled to the backbone of a thermoreversible hydrogel, agarose. The amount of LN-1 coupled per milliliter of gel was optimized *in vitro* to be $\sim 60 \mu\text{g/mL}$.¹⁶ Briefly, LN-1 (GIBCO, Rockville, MD) was covalently coupled onto SeaPrep agarose gel (FMC, Rockland, ME) through carbonyldiimidazole (CDI; Sigma-Aldrich, St. Louis, MO) chemistry.¹⁶⁻¹⁸ As measured by the Bio-Rad (Hercules, CA) protein assay, the efficiency of LN-1 coupling to agarose was approximately 20%, and the resulting amount of LN-1 covalently coupled to agarose was $67 \mu\text{g/mL}$ of 1% agarose gel.

Slow release of NGF is desired for two reasons. First, if NGF were simply mixed into our hydrogel bridges, it would diffuse away from the nerve gap in a matter of hours.¹³ Second, we wanted to set up diffusion gradients that will attract the regenerating nerve into the bridge and continue to grow across its length; we believe that slow release of NGF is essential to that end. In addition, when NGF release persists in high concentrations within the bridge materials, its chemoattractant qualities may interfere with axonal regeneration. A novel lipid microtubule (LMT)-based system of 1,2-bis(tricoso-10,12-diyonyl)-

sn-glycero-3-phosphocholine (DC_{8,9}PC; Avanti Polar Lipids, Alabaster, AL) was fabricated by ethanol deposition and used for NGF slow release.^{16,19} The LMTs had an average length of $40 \mu\text{m}$ and a luminal diameter of $0.5 \mu\text{m}$.^{16,20} NGF was loaded at a protein:lipid ratio of 1% (w/w) by hydration of 10 mg of lyophilized LMTs with 1 mL of phosphate-buffered saline (PBS) containing $100 \mu\text{g}$ of NGF. The NGF-loaded LMTs were embedded into agarose or LN-1-coupled agarose by mixing LMTs with agarose or LN-agarose solution at 37°C . NGF is slowly released by diffusion from the open ends of the LMTs. We have developed a mathematical model that predicts the duration of release of NGF, when its diffusion coefficient in saline, and agarose hydrogel, is known. Using this model, we loaded sufficient NGF into our LMTs to allow for physiological concentrations ($>5 \text{ ng/mL}$) of NGF to be released for at least 42 days *in vitro*.²¹ Our previously published studies have shown that the bioactivity of NGF showed no degradation when released from LMTs for 7 days at 37°C *in vitro*.¹⁶ As it usually takes 1 to 2 months for peripheral nerve to regenerate across a 10-mm-long gap,^{22,23} the 42-day release of NGF was deemed appropriate for this specific application. We have previously reported that proteins released from LMTs undergo a burst release for the first 2–3 days, and then the release profile tends to zero-order.²¹

Preparation of polymer guidance channels. Polysulfone guidance channels (Koch Membrane Systems, Wilmington, MA) used in this study had an inner diameter of 1.6 mm, an outer diameter of 3.2 mm, and a length of 12 mm. The molecular weight cutoff (MWCO) of the guidance channels is 50,000. For sterilization, the polysulfone guidance channels were immersed in 70% ethanol solution for 48 hours, dried under a laminar flow hood, and washed with sterilized PBS. The implants were separated into seven categories as described in Table 1. Control group I (PBS) contained channels filled with PBS, pH 7.4 (GIBCO). Control group II (AG) contained channels filled with 0.5% (w/v) plain (unmodified) agarose in PBS. Control group III (AG-PBS) contained channels filled with 0.5% (w/v) agarose gel embedded with 10% PBS-loaded LMTs (10 mg of lipid per 1 mL of gel). The positive control group IV (AU) consisted of 10-mm nerve autografts, which were resected from the rat sciatic nerve being bridged. Experimental group I (AG-LN) contained channels filled with 0.5% (w/v) laminin-agarose gel. Experimental group II (AG-NGF) was filled with 0.5% agarose gel embedded with 1% NGF-LMTs (10 mg of lipid per 1 mL of PBS, $10 \mu\text{g}$ of NGF per 1 mg of lipid). Experimental group III (AG-LN-NGF) contained channels filled with 0.5% laminin-agarose gels embedded with 1% NGF-LMTs (10 mg of lipid per 1 mL of PBS, $10 \mu\text{g}$ of NGF per 1 mg

TABLE 1. DESCRIPTION AND NOTATION FOR EXPERIMENTAL AND CONTROL GROUPS

Group	Notation	Components
Control group I (negative)	PBS	Phosphate-buffered saline
Control group II (negative)	AG	Plain agarose gel in PBS solution
Control group III (negative)	AG-PBS	Agarose gel embedded with PBS-LMTs
Control group IV (positive)	AU	Autograft, which was resected from the rat sciatic nerve
Experimental group I	AG-LN	LN-1-coupled agarose gels
Experimental group II	AG-NGF	Agarose gel embedded with NGF-LMTs
Experimental group III	AG-LN-NGF	LN-1-coupled agarose gels embedded with NGF-LMTs

Abbreviations: AG, Agarose; AU, autograft; LMT, lipid microtubule; LN, laminin; NGF, nerve growth factor.

of lipid). As SeaPrep agarose gels at 17°C, the agarose solutions were filled into the channels in their liquid state at room temperature, using a 20-gauge needle and a 1-cm³ syringe, and gelled at 4°C for 30 min. The gel-containing polymer guidance channels were stored at 4°C until implantation *in vivo* shortly after gelation.

In the LN-1-containing groups, LN-1 was three-dimensionally presented in the tubes at the concentration of 34 µg/mL of 0.5% (w/v) agarose scaffolds as assessed by the Bio-Rad protein assay. In the NGF-containing groups, NGF was loaded in the tubes at an initial concentration of 100 µg/mL, and the NGF was slowly released from the LMTs.^{16,21}

Implantation of tissue-engineered scaffolds

Introduction of tissue-engineered scaffolds across 10-mm sciatic nerve gap. Adult Fischer male rats (Harlan Sprague Dawley, Indianapolis, IN) weighing between 200 and 300 g were used as experimental animals. Seventy animals were divided into 7 groups with implants as described in Table 1. Specifically, 13, 12, 13, 5, 9, 12, and 6 animals were implanted for PBS, AG, AG-PBS, AU, AG-LN, AG-NGF, and AG-LN-NGF groups, respectively. Rodents were anesthetized with sodium pentobarbital solution (60 mg/kg; Abbott Laboratories, Chicago, IL). After shaving the left thigh region and applying povidone-iodine (Betadine; West Chemicals, Long Island City, NY), a 15-mm incision was made along the femoral axis. The thigh muscles were separated and the sciatic nerve was dissected free. Using sharp microscissors under a surgical microscope, the nerve was transected and a 10-mm segment of nerve was explanted. The proximal and distal stumps were secured 10 mm apart in 12-mm-long polysulfone channels carrying the experimental or control gel formations or autografts, using a 10-0 Nylon monofilament suture (Ethicon, Piscataway, NJ). The muscles were then closed with a 4-0 plain gut suture and the skin was closed with 4-0 silk sutures (Ethicon). Marcaine (0.5%; Abbott Laboratories) was

added to the dose of 0.3 mL per rat to the surgery site for pain relief. National Institutes of Health (NIH, Bethesda, MD) guidelines for using experimental animals were followed both pre- and postoperatively. The resected 10-mm nerve was fixed in 4% paraformaldehyde and prepared for histological analysis to evaluate the native nerve before injury.

Evaluating regeneration

Histological analysis. Two months postimplantation, an overdose of sodium pentobarbital was administered, followed by intracardial perfusion with saline and cold 4% paraformaldehyde. The operative site was reopened and the polymer guidance channels/autografts were explanted. Each autograft was identified by the 10-0 nylon suture landmarks and a 12-mm segment was resected (1 mm on either side of the autograft was included). The explanted guidance channels/autografts were stored in 4% paraformaldehyde, and embedded in paraffin. Three-micron serial sections were cut, mounted on slides, and stained with osmium tetroxide, followed with toluidine blue. Nerve regeneration was evaluated by parameters that included (1) the success rate of regeneration, defined as the percentage of channels with successful cable formation (cable-like continuous tissue formation within the tubes containing myelinated axons), (2) the total number of myelinated axons, and (3) the density of myelinated axons (number of myelinated axons per unit area) at the midpoint of the channel/autograft. Axon counts were performed under an optical microscope (×600; Nikon, Tokyo, Japan). The area of regenerated nerve cable was quantified under a microscope equipped with a digital camera (Optronics, Goleta, CA) connected to a computer running Image-Pro Express software (MediaCybernetics, Carlsbad, CA). In addition to the groups with transplants, the number of myelinated axons and density of myelinated axons (axons per unit area) of five native nerve segments were also determined for reference, and denoted as normal.

Gastrocnemius muscle weight. Gastrocnemius muscle mass is proportional to the degree of sciatic nerve innervation,²⁴ and provides evidence for functional activity of sciatic nerves.²⁴ After 2 months of implantation, the gastrocnemius muscles from both the left and right sides of the animals were collected and weighed. The relative gastrocnemius muscle weight (RGMW), defined as the ratio of the gastrocnemius muscle weight from the experimental (right) side to that of the normal (left) side, was used to evaluate the “functional” consequence of sciatic nerve regeneration. In addition to the groups with transplants, the RGMW of five rats that did not undergo any treatment was also determined for reference, and denoted as normal.

Walking track analysis. Walking track analysis was used as another measure of functional muscle reinnervation. The paw prints from the experimental and control hind limbs were compared. The sciatic functional index (SFI) derived from the walking track analysis has been widely adopted as an indicator of functional nerve recovery.^{25,26} As the SFI value approaches zero, the corresponding functional recovery is better. Preoperatively, the right hind paws of the rats were painted with trypan blue dye, and the right hind paw prints were recorded by training the animal to walk in a box. One and 2 months after surgery, the same procedure was repeated and the right hind paw prints were again recorded. Three parameters were derived from the paw prints: print length (PL), toe spread (TS; distance from toe 1 to toe 5), and intermediate toe spread (IT; distance from toe 2 to toe 4). The parameters from paw prints taken before and after surgery were considered to be normal and experimental, re-

spectively. The sciatic functional index was calculated by using the following formula²⁷:

$$\text{SFI} = -38.8 \left(\frac{\text{Experimental PL} - \text{Normal PL}}{\text{Normal PL}} \right) + 109.5 \left(\frac{\text{Experimental TS} - \text{Normal TS}}{\text{Normal TS}} \right) + 13.3 \left(\frac{\text{Experimental IT} - \text{Normal IT}}{\text{Normal IT}} \right) - 8.8$$

This formula was developed by De Medinaceli *et al.*²⁸ and modified by Bain *et al.*²⁷ (For a detailed description, please see the review by Varejao *et al.*²⁵)

Statistical analysis

The data were expressed as means \pm SEM. Single-factor analysis of variance (ANOVA) with pairwise comparisons between groups (each comparison at two-sided 5% level of significance) was performed. A *p* value less than 0.05 was considered statistically significant.

RESULTS

On visual inspection at the time of guidance channel explantation, all the polysulfone channels were covered with a thin fibrous tissue, and no swelling, collapse, or broken channels were observed. Depending on the scaffold formulation used to fill the guidance channels, different percentages of implanted channels had successful nerve cable formation with myelinated axons. The num-

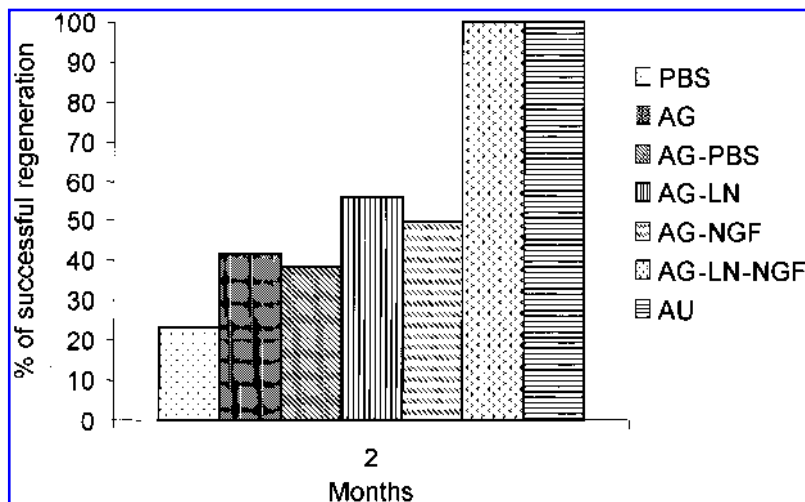


FIG. 1. Graph showing the percent success rate of regeneration in control and tissue-engineered scaffolds. The success rate of regeneration was determined as the number of channels with continuous nerve cable containing myelinated axons divided by total number of channels implanted.

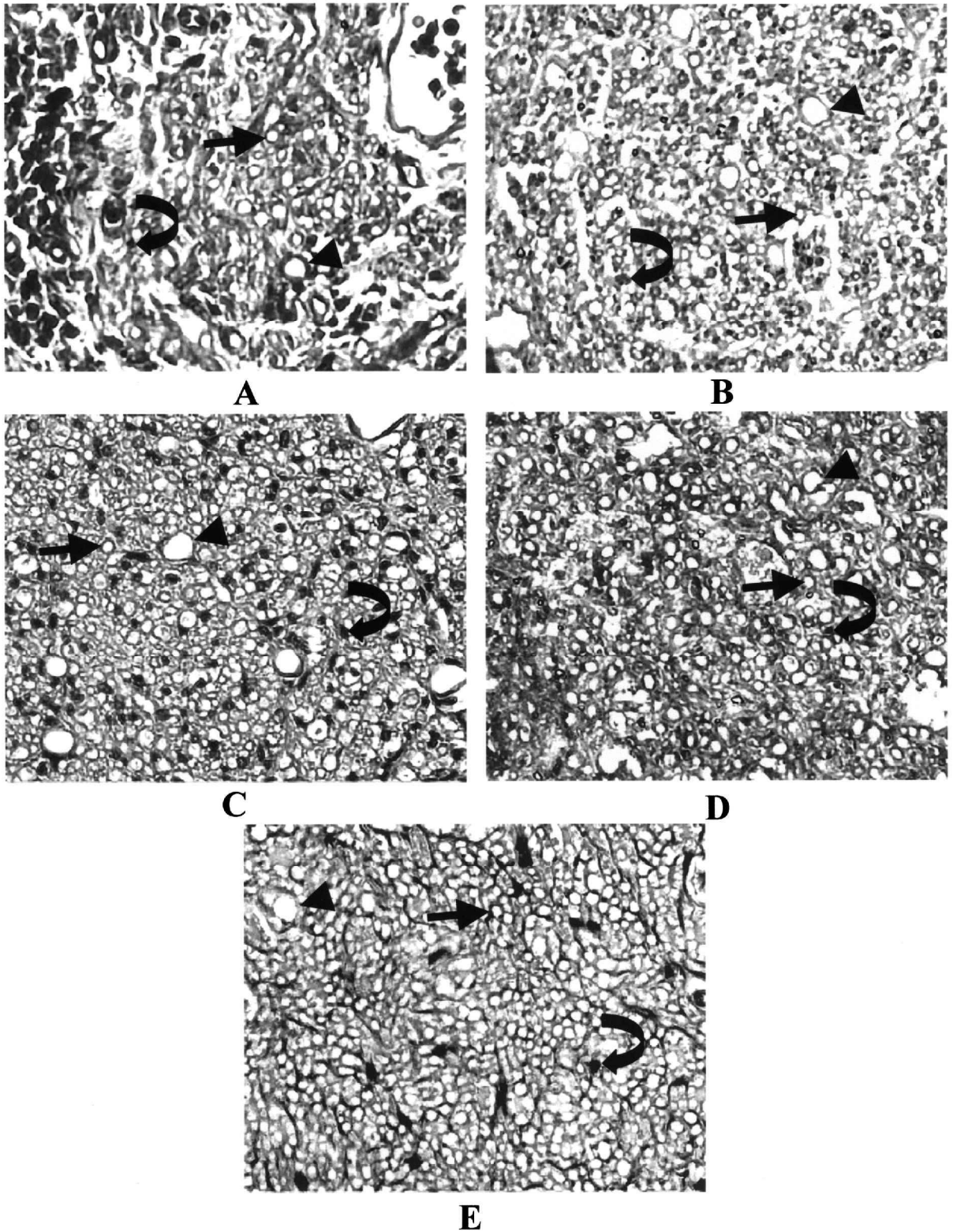


FIG. 2. Light micrograph of nerve cross-sections at the midpoint of the guidance channel filled with (A) PBS; (B) agarose; (C) laminin-coupled agarose embedded with NGF-loaded lipid microtubules; and (D) nerve autografts. (E) Cross-section of a native sciatic nerve. Arrows point to myelinated axons; arrowheads point to vascular grafts; curved arrows point to nuclei of Schwann cells.

ber of animals with regenerated cables was 3, 5, 5, 5, 6, and 6 separately, corresponding to groups PBS, AG, AG-PBS, AU, AG-LN, AG-NGF, and AG-LN-NGF. As shown in Fig. 1, when the number of animals implanted in each group is taken into account, the percentage of animals with successful cable formation containing myelinated axons was 26% (PBS), 42% (AG), 37% (AG-PBS), 100% (AU), 55.5% (AG-LN), 50% (AG-NGF), and 100% (AG-LN-NGF). The PBS group had the lowest success rate of cable formation, whereas the AG-LN-NGF and AU groups had the highest success rate of cable formation. Relatively higher numbers of animals were implanted for the negative control groups compared with experimental groups to ensure that at least three animals had successfully regenerated cables in every group to allow a comparative histological analysis between control and experimental groups.

Photographs of toluidine blue-stained cross-sections at the midpoint of nerve guidance channels/autografts are shown in Fig. 2A–E. The gross structure of the regenerated nerves was similar to that of normal sciatic nerves. Regenerated axons were packed in bundles to form fascicles, and nuclei from Schwann cells clearly surrounded the regenerated axons. There were abundant blood vessels in the endoneural areas of regenerated nerves. No evidence of any remnant agarose was found either on macroscopic observation at the time of explantation, or on microscopic examination of histologically processed, transverse or longitudinal cross-sections of the tubes.

The total number of myelinated axons and the density of myelinated axons were significantly higher in positive control and experimental groups (AU, AG-LN, AG-NGF, and AG-LN-NGF) than in the negative control groups

(PBS, AG, and AG-PBS) (Figs. 3 and 4). The total number of myelinated axons and the density of myelinated axons in AG-LN-NGF scaffolds were also significantly higher than were observed in AG-LN and AG-NGF scaffolds. No significant difference in the total number of myelinated axons, or in the density of myelinated axons, was observed between the best performing scaffold, AG-LN-NGF, and the autograft, AU. The AG-LN-NGF scaffolds contained 5501 ± 1550 myelinated axons at a density of $17,425 \pm 1388$ myelinated axons/mm². In comparison, the native nerve contained 8638 ± 1356 myelinated axons at a density of $17,118 \pm 664$ myelinated axons/mm². The autograft transplants contained 5743 ± 655 myelinated axons at a density of $16,983 \pm 1601$ myelinated axons/mm². These results imply that at 2 months, both our tissue-engineered scaffolds and autografts had myelinated axon density comparable to native nerve, but with 40% fewer myelinated axons.

The relative gastrocnemius muscle weight determination showed that RGMW increased in the order of PBS, AG, AG-PBS, AG-LN, AG-NGF, AG-LN-NGF, and AU (Fig. 5). Compared with that of the negative control groups (PBS, AG, and AG-PBS), RGMW in the experimental groups (AG-LN, AG-NGF, and AG-LN-NGF) and positive control group (AU) was significantly higher. No significant difference in RGMW was observed among the AG-LN, AG-NGF, AG-LN-NGF, and AU groups.

One month postoperatively, no significant difference in SFI values was observed among any of the groups tested (Fig. 6). At 2 months postoperatively, SFI values in the AG-LN and AG-NGF groups decreased slightly, whereas SFI values in the AU and AG-LN-NGF groups decreased significantly compared with the negative con-

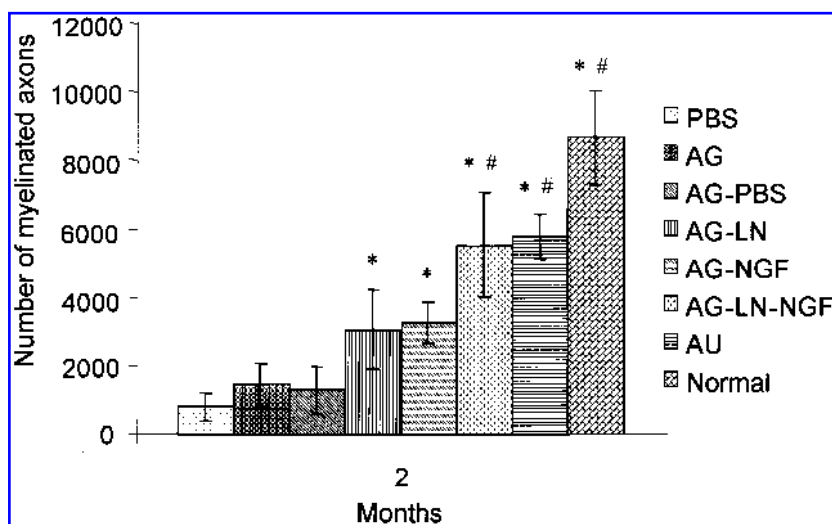


FIG. 3. Total number of myelinated axons from sections at the midpoint of the channel/autograft for control and experimental groups with successful cable formation at 2 months. *Statistically significant, higher percentage ($p < 0.05$) compared with PBS, AG, and AG-PBS. #Statistically significant, higher percentage ($p < 0.05$) compared with AG-LN and AG-NGF. Error bars denote the SEM.

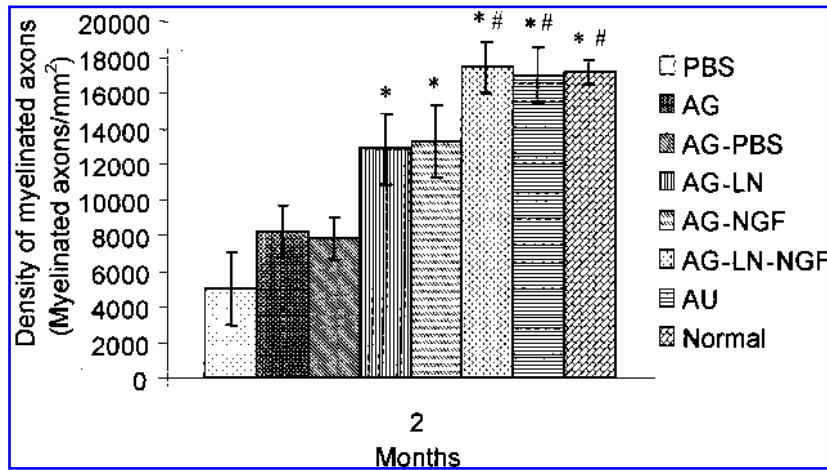


FIG. 4. Number of myelinated axons per unit area calculated from sections at the midpoint of the channel/autograft for control and experimental groups with successful cable formation at 2 months. *Statistically significant, higher percentage ($p < 0.05$) compared with PBS, AG, and AG-PBS. #Statistically significant, higher percentage ($p < 0.05$) compared with AG-LN and AG-NGF. Error bars denote the SEM.

trol PBS, AG, and AG-PBS groups ($p < 0.05$) (Fig. 6). No significant difference in SFI values was observed among the AG-LN, AG-NGF, AG-LN-NGF, and AU groups.

DISCUSSION

In development, axons are guided to their targets by a combination of substrate-mediated cues from extracellular matrix or cell adhesion proteins, and diffusible cues that include neurotrophic factors.²⁹⁻³¹ Extracellu-

lar matrix proteins affect cell interactions during the development, maintenance, and regeneration of the nervous system.³² Neurotrophic factors support growth, differentiation, and survival of neurons in the nervous system.³³ LN-1 and NGF promote both the survival and growth of axons in the peripheral nervous system (PNS).³³ We have designed a tissue-engineered agarose-based scaffold with both substrate- and diffusion-mediated cues for peripheral nerve regeneration. In the current study we report the efficacy of these scaffolds in an *in vivo* nerve regeneration model in rodents. We decided to use a 10-mm nerve gap, consistent with that used in the majority of studies

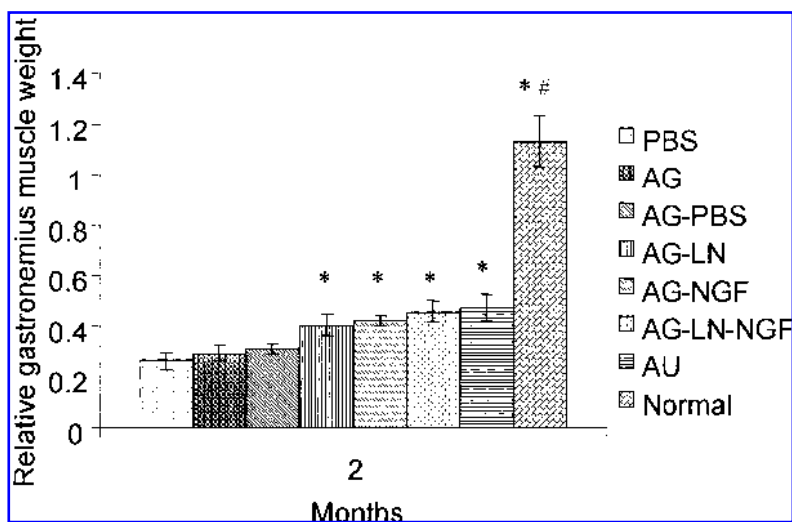


FIG. 5. Relative gastrocnemius muscle weight (RGMW) for animals with successful cable formation at 2 months. RGMW is defined as weight of experimental side muscle (right) divided by weight of control side muscle (left) at the time of explanation. *Statistically significant, higher percentage ($p < 0.05$) compared with RGMW of PBS, AG, and AG-PBS. #Statistically significant, higher percentage ($p < 0.05$) compared with AG-LN, AG-NGF, AG-LN-NGF, and AU. Error bars denote the SEM.

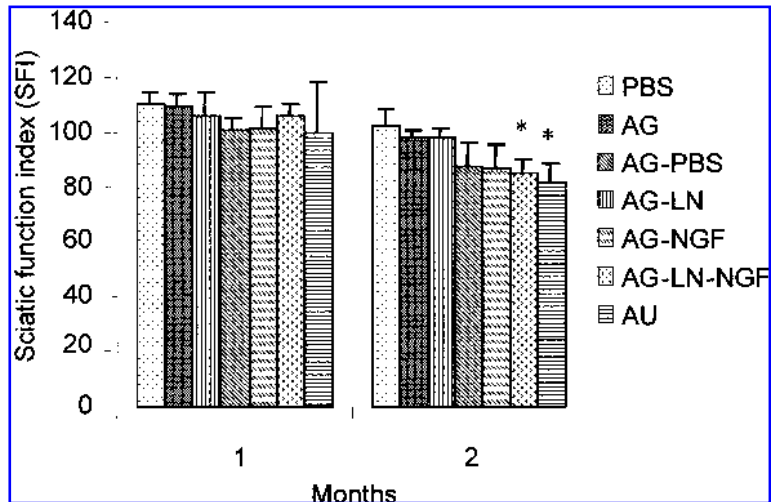


FIG. 6. Absolute value of the sciatic functional index (SFI) for the control and experimental animals. *Statistically significant, lower value ($p < 0.05$) compared with the negative control groups (PBS, AG, and AG-LMC). Error bars denote the SEM.

testing alternative strategies for peripheral nerve repair. In this report, we demonstrate that tissue-engineered scaffolds perform comparably to the current clinical “gold standard”—autografts.

Semipermeable polysulfone guidance channels are commercially available and are convenient vehicles for the introduction of tissue-engineered hydrogel scaffolds to the site of nerve injury. We recognize, of course, that although polysulfone channels elicited only minor fibrous tissue reaction, ideally, bioresorbable guidance channels would be better carriers of our tissue-engineered scaffolds.

As saline-filled guidance channels are dependent on the native fibrin cable formation for regeneration, it is not surprising that they performed the worst. We hypothesize that agarose gels either aid fibrin cable formation, or provide extracellular cues that augment native Schwann cell-mediated regenerative signals, especially in the longer gaps, and thus perform better than PBS-filled channels.

We have designed a two-component system in which substrate-bound neurite-stimulatory extracellular matrix (ECM) molecule LN-1 is combined with a micron-scale, drug delivery system for sustained release of neurotrophic factor NGF. When covalently coupled LN-1 and sustained delivery of NGF were introduced into our scaffolds together, a synergistic improvement was observed. We have reported earlier that covalent coupling of LN-1 to agarose is necessary for enhanced activity.¹⁶ Others have demonstrated that a single-dose administration of NGF dramatically dissipates NGF from the site of injury within 24 h.¹³ These reports are consistent with our observations in this study suggesting that both covalent coupling of LN-1 and sustained delivery of NGF are

important for successful regeneration. However, a comparison of the number and density of myelinated axons regenerated across our best tissue-engineered scaffold (AG-LN-NGF), autograft and native nerve, reveals that although the density of myelinated axons is comparable, the AG-LN-NGF scaffold and the autograft contain a significantly lower number of myelinated axons at 2 months. Although this lower number is not ideal, several studies demonstrate that significant improvement in function is observed with relatively few functioning fibers in the nervous system.^{34,35}

In our functional assays, the RGMW of the experimental groups (LN-1- and NGF-containing scaffolds) was significantly higher than that of the control groups. No significant difference in RGMW was observed between the autograft group and the group that contained both LN-1 and NGF.

The absolute values of SFI from the experimental groups (all LN-1- and NGF-containing scaffolds) were lower than those from the control groups. No significant difference in SFI was observed in comparing the LN-1 group, NGF group, the group with both LN-1 and NGF, and the autograft group. However, only the autograft group and the group with both LN-1 and NGF showed significantly decreased SFI values, compared with the PBS control group. Therefore, the LN-1- and NGF-containing scaffolds promoted significant functional muscle recovery and performed comparably to autografts.

In both RGMW and SFI studies, the functional improvement was not as dramatic as that observed histologically. The likely explanation is that regenerating axons take longer than 2 months to traverse the distance from the distal stump to reinnervate the target distal musculature.

The precise mechanisms by which LN-1- and NGF-containing scaffolds enhance peripheral nerve regeneration are not clear. One hypothesis is that the LN-1- and NGF-containing scaffold may provide the necessary early cues to promote nerve sprouting, and then act as a bridge to augment the fibrin cable, thereby supporting Schwann cell invasion and axon regeneration.

Our scaffold design introduces LN-1 and NGF simultaneously to the regenerating nerve stump. As LN-1 does not form a gel on its own, Matrigel, an LN-1-rich gel derived from a mouse sarcoma, has been used in some *in vitro* and *in vivo* studies. Matrigel yields mixed results, and can never be used clinically as it is poorly characterized and is filled with inhibitory cytokines. Other studies attempting to use NGF alone to stimulate peripheral nerve repair have also not been successful. In this sense, ours is the first study combining the presentation of LN-1 in three-dimensional scaffolds and slow release of NGF to clearly demonstrate a successful outcome.

Others have used tissue-engineering strategies to promote peripheral nerve regeneration *in vivo*.^{36,37} Fibrin gels incorporated with laminin and N-cadherin peptides facilitated nerve regeneration across a 4-mm gap in rat dorsal root.³⁶ Magnetically aligned collagen gels promoted regeneration of the sciatic nerve in mice across a 6-mm gap.³⁷ Although these approaches are promising, few have used autograft controls, so it is difficult to assess their clinical potential. We are aware, however, that some studies have previously reported equivalence to autografts in repairing 10-mm nerve gaps.^{38,39} In this study, we chose the 10-mm-long gap as the logical first step to test our new scaffold formulations *in vivo* in rodents. We believe that our approach is easily scalable to larger nerve gaps and to further test our strategy, our follow-up studies will focus on developing scaffolds that perform as well as or even better than autografts in repairing larger nerve gaps (>25 mm).

Our tissue-engineered scaffolds perform comparably to autografts in bridging 10-mm peripheral nerve gaps *in vivo* in rodents, and have the potential to be used as alternative strategies to autografts for repairing peripheral nerve injury clinically.

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