Laminin-coated poly(L-lactide) filaments induce robust neurite growth while providing directional orientation

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Abstract: Cellular channels during development and after peripheral nerve injury are thought to provide guidance cues to growing axons. In tissue culture where these cues are absent, neurites from dorsal root ganglion neurons grow with a radial distribution. To induce directional axonal growth and to enhance the rate of axonal growth after injury, we have designed microfilaments of poly(L-lactide). We demonstrate that dorsal root ganglia grown on these filaments *in vitro* extend longitudinally oriented neurites in a manner similar to native peripheral nerves. The extent of neurite growth was significantly higher on laminin-coated filaments compared with uncoated and poly-L-lysine–coated filaments. As high as 5.8 ± 0.2 mm growth was observed on laminin-coated filaments compared with 2.0 ± 0.2 mm on uncoated and 2.2 ± 0.3 mm on poly-L-lysine–coated filaments within 8 days. Schwann cells were found to grow on all types of filaments. They were, however, absent in the leading edges of growth on laminin-coated filaments. Photolysis of Schwann cells caused a significant reduction in the neurite length on all types of filaments. Laminin-coated filaments, however, induced significantly longer neurites compared with uncoated and/or poly-L-lysine–coated filaments even in the absence of Schwann cells. Our results suggest that laminin-coated poly(L-lactide) filaments are suitable for inducing directional and enhanced axonal growth. Implants designed by arranging these microfilaments into bundles should aid regenerating axons by providing guidance cues and channels to organize matrix deposition, cell migration, axon growth, and improve functional recovery. © 2000 John Wiley & Sons, Inc. J Biomed Mater Res, 51, 625–634, 2000.

Key words: PLLA filaments; bioresorbable polymers; dorsal root ganglia; laminin; PNS regeneration

INTRODUCTION

Axons can spontaneously regenerate over relatively short distances (less than 5 mm) in the adult peripheral nervous system (PNS) but not at all in the adult central nervous system. Even though regeneration occurs after injury to the adult PNS, it does not always result in functional recovery, due primarily to misdirection of regenerating axons toward an inappropriate target. When the nerve is separated by a gap greater than 1 cm in length, the lack of specific guidance cues can lead axons to grow backwards into the proximal nerve stump, enter an inappropriate endoneurium, or form neuromas.[1,2] In all these conditions, injured axons can take several months to regenerate, by which time degenerative changes can occur and lead to atrophy of target organs. To increase the prospects of axonal regeneration and functional recovery, numerous strategies have been used, such as implantation of autografts, allografts, xenografts, and Schwann cell-filled tubes.[3–5] Although autografts are the clinically ideal graft type, they pose the disadvantages of limited availability and the sacrifice of a healthy nerve, which causes permanent denervation of the donor site. Axonal regeneration through Schwann cell-filled tubes has shown success in a number of animal models; however, the isolation and expansion of Schwann cells for the treatment of nerve injury in humans have proven to be difficult. Allografts and xenografts have also been used but require immunosuppression to reduce the prospects of graft rejection and have relatively poor success rates.[6] These disadvantages have led to the development of artificial nerve grafts.

Synthetic, nondegradable grafts have been widely used to study neural regeneration. Early studies used empty or saline-filled silicone tubes to aid nerve repair over distances of up to 1 cm.[7,8] Although these tubes have some advantages over the above-mentioned

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grafts, their use is limited. Because they are nonbiodegradable, they remain *in situ* as foreign objects, and scar tissue often envelops the tubes.[9,10] Unlike silicone tubes, bioresorbable grafts show excellent biocompatibility and biodegradability. They not only provide initial structural support for regenerating axons but also degrade over time[11–13] and provide space for the growing axons. In addition, they obviate the need for second surgery and the long-term deleterious effects observed with synthetic nonbiodegradable grafts, such as chronic contraction, pain, foreign body reaction, etc.[14,15] Several studies have reported the good acceptance of these materials by the body.[16–18] However, a few others have reported either transient or mild to moderate inflammatory reactions to biopolymer implants.[19,20] Although the end products of degradation are nontoxic, the increased acidity and the degradation products by themselves may have a role in the inflammatory reaction.[18–21] Bioresorbable polymer tubes have also been widely used to promote axonal regeneration. Although axonal regeneration does occur inside these tubes, the growth is limited to distances of up to 1.0 cm, and functional recovery is poor.

To enhance axonal regeneration, particularly across gaps longer than 1 cm, growth factors,[22] fibrin,[23,24] and extracellular matrix molecules, such as collagen, laminin, and fibronectin,[25–28] have been added to the lumen of the tube before implantation. These substances are thought to enhance the migration of non-neuronal cells into the chamber to form a cable that extends between the nerve ends when the endogenous intratubular matrix fails to form. The cable then serves as a scaffold to support the growth of axons across the gap,[29] Scaffold organization and the prospect of functional regeneration could be increased further by providing longitudinal alignment and guidance channels to the matrix within the tube.[29] To provide the longitudinal organization, guidance channels, and a growth substratum for either Schwann cells or axons, we have fabricated fine filaments from the bioresorbable polymer poly(L-lactide) (PLLA).

PLLA by itself or copolymerized with other bioresorbable polymers, such as poly(ε-caprolactone) or polyglycolic acid has been used to fabricate tubular nerve guides for implantation after PNS injury.[30–34] The PLLA filaments fabricated for our study are fine, flexible, and strong. These filaments can be bundled inside a tube to provide a longitudinally organized growth substrate, with defined space in between the filaments to allow the migration of non-neuronal cells and axons. When placed between the severed ends, this structure should act as an acellular cable, directionally orienting neurotrophin diffusion, matrix formation, and cellular and axonal growth within the tube, thereby enhancing regeneration.

In the present study, we examined the suitability of these filaments to support directional growth of Schwann cells and axons using dorsal root ganglia (DRG) *in vitro*. The results demonstrate that the newly designed filaments orient the growth of Schwann cells and neurites along the longitudinal axis of the filament. In addition, the extent of neurite outgrowth significantly increased when the filaments were coated with laminin, compared with uncoated and poly-L-lysine (PLL)–coated filaments. Schwann cells were not necessary for neurite growth on laminin-coated filaments, but their presence increased the extent of neurite growth on all of the three types of filaments.

MATERIALS AND METHODS

Fabrication of PLLA filaments and surface modification

Filament fabrication

PLLA filaments were fabricated by a melt extrusion process. Briefly, the polymer pellets were loaded into the extruder (Alex James, Charleston, SC) and melted. The molten polymer was then forced through a spinneret having a hole 1.5 mm in diameter and dropped through air to a heated spinning bobbin (60°C, 60 rpm). The filament thus formed was then stretched to 4:1 ratio to increase crystallinity and tensile strength, and stored in a desiccator until use.

Modification of the filament surface

PLLA filaments were cut into 1-cm lengths, rinsed with phosphate buffered saline (PBS) (pH 7.2), and coated with either laminin (GIBCO-BRL; 10 μg/mL in PBS) overnight at 4°C (rocking) or precoated with PLL (Sigma, St. Louis, Mo; 50 μg/mL in PBS) for 2 h at room temperature followed by incubation in laminin overnight at 4°C. The filaments were then washed 3 times with PBS and used for the experiments.

Quantitation of laminin by indirect enzyme linked immunosorbent assay (ELISA)

The amount of laminin bound to filaments coated with laminin or precoated with PLL followed by laminin was determined by ELISA. Uncoated and PLL-coated filaments served as controls. Filaments (4 × 1 cm) from each treatment were placed in 1.5-mL Eppendorf tubes and blocked with 1% bovine serum albumin (BSA) (250 μL/tube) in Tris-buffered saline for 1 h, followed by incubation with antibodies to rat laminin (GIBCO-BRL; diluted 1:500) (200 μL) for 2 h and anti-rabbit IgG (F$_c$) (H + L) conjugated to alkaline phosphatase (AP) (200 μL) for 1 h. The reaction was developed using *p*-nitrophenyl phosphate, disodium (Sigma; 3 mM) in carbonate buffer, pH 9.6 (100 mM Na$_2$CO$_3$ and 5 mM MgCl$_2$) (150 μL), and the reaction was terminated after 30 min using 0.5N NaOH (50 μL). The colored product was then transferred to a 96-well microtiter plate, and ab-

sorbance was read at 405 nm using the microplate reader (Bio-Tek instruments, Winouskie, VT). The relative amount of laminin bound to the filaments was measured from the densitometric readings against a standard curve obtained for laminin plated onto a 96-well microtiter plate at concentrations ranging from 1 ng to 1 μg per well.

The stability of laminin binding to PLLA filaments (1 cm) and PLL-precoated PLLA filaments was examined by suspending the laminin-coated filaments in Dulbecco's modified Eagle's MEM (DMEM) (GIBCO-BRL) containing 10% fetal calf serum (FCS) (GIBCO-BRL) and incubating in a CO_2 incubator at 37°C for various time periods (0, 1, 2, 4, and 8 days). The filaments were collected at each time point and stored at −80°C until use. The amount of laminin remaining bound to the filaments was estimated using ELISA as described above.

DRG culture and neurite growth

Isolation of DRG

DRG were isolated from newborn rat pups (Sprague-Dawley). The pups were anesthetized by hypothermia, decapitated, and the dorsal skin was excised. After performing a laminectomy and removing the spinal cord, DRG were isolated and digested in 1% collagenase (Sigma) in Hanks' balanced salt solution (HBSS) for 20–30 min. After digestion, the DRG were washed with HBSS and resuspended in DMEM containing 10% FCS and 20 ng/mL β-nerve growth factor (NGF) (GIBCO-BRL) and cultured on the PLLA filaments with different treatments.

Culturing DRG on PLLA filaments

PLLA filaments (2-cm length) were treated with 10 X antibiotic solution, penicillin G (100 U/mL), and streptomycin sulfate (100 μg/mL) (GIBCO-BRL) for 2 h at room temperature, followed by rinsing with PBS and air-drying. The filaments were then attached to the bottom of the 60-mm culture dishes by carefully applying very small amounts of quick gel (cyanoacrylate ester) to their tips, preventing spread of gel along the filaments. The dishes were previously coated with 1% BSA in PBS for 2 h, washed, and dried before attaching the filaments onto them. Approximately 8–10 filaments were attached per plate, very close to each other in a parallel array. The array was then rinsed with PBS, coated with PLL (50 μg/mL) in PBS for 2 h at room temperature, and then with laminin (10 μg/mL in PBS) overnight at 4°C. Uncoated and PLL-coated filaments once again served as controls. Filaments with different treatments were then covered with melted agarose (0.3%) in DMEM and 10% FCS. After agarose solidification, a small well was made at the center over the array by aspirating off the agarose. Freshly isolated DRG suspended in 10 μL of DMEM + 10% FCS and 20 ng/mL of β-NGF were placed inside these wells (1 to 2 per well). The culture dishes were then carefully transferred to a 37°C incubator with 4% CO_2 and 95% humidity and cultured for 3 to 8 days.

Photolysis of Schwann cells

Freshly isolated DRG were cultured on PLLA filaments as described above. After a 24-h attachment period, Schwann cells were photolyzed by labeling with 5-bromodeoxyuridine (5-BrdU) and Hoechst 33258.[35] The cultures were incubated in a medium containing 50 μM 5-BrdU (Aldrich, St. Louis, MO) for 48 h to ensure that all the cells divided at least once and incorporated 5-BrdU. The cultures were then rinsed with DMEM and incubated in medium containing 5 μg/mL Hoechst 33258 (Molecular Probes, Eugene, OR) for 3 h. After replacing the medium with DMEM + 10% FCS, the cultures were exposed to light using a fluorescent lamp (60 Watts) 6 cm above the culture dish for 20 min and transferred back to the CO_2 incubator at 37°C and grown for another 5 days. Control cultures without photolysis were incubated for the same period of time under similar conditions.

Indirect immunocytochemistry

Neurite growth and Schwann cell growth on PLLA filaments with different treatments was visualized by immunocytochemical labeling for neurofilament protein and p75 (low affinity NGF receptor), respectively. DRG grown on PLLA filaments for 8 days were fixed using 4% paraformaldehyde in 0.1M phosphate buffer (pH 7.2) for 1 h at 4°C. After rinsing with PBS several times, the specimens were blocked with 5% normal goat serum in dilution buffer (25 mM Tris-HCl, 300 mM NaCl, 0.3% Triton X-100, 0.5 mg/mL BSA, 0.01% Thimerosal, pH 7.2) for 1 h and then labeled with either neurofilament antibody (SMI-32, Sternberger monoclonals, Baltimore, MD; diluted 1:10,000) or p75 antibody (Promega, Madison, WI; diluted 1:1000) for 18 h. For neurofilament detection, the specimens were incubated with biotinylated anti-mouse IgG (H+L) (Jackson Immuno Research Laboratories, Inc.; diluted 1:600) for 1 h and further processed using Vectastain "Elite" ABC reagents (Vector Laboratories, Inc.) and developed using diaminobenzidine (0.02%) and hydrogen peroxide (0.003%) in Tris-Cl, pH 7.2. For p75 detection, the samples were incubated with anti-rabbit IgG conjugated to AP (Promega; 1:7,500) for 1 h and then developed using nitro blue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate in AP buffer (100 mM Tris-HCl, 5 mM $MgCl_2$, 100 mM NaCl, pH 9.5). All incubations were performed at room temperature in a humid chamber. After staining, the specimens were mounted with glycerol, viewed under Zeiss Axiophot, and photographed using a digital video camera.

Scanning electron microscopy

DRG were cultured for 3 days on PLLA filaments coated with PLL and laminin and fixed using 2% paraformaldehyde and 0.5% glutaraldehyde in phosphate buffer. The specimens were osmicated (1% OsO_4) on ice for 1 h and dehydrated through a graded series of alcohol. After critical point drying and sputter coating with carbon, the specimens were mounted on carbon stubs and imaged with a JEOL 840-A, Peabody, MA scanning electron microscope at 10 KeV, 39–43 mm working distance, and 400 to 1000 magnifications.

Quantitative analysis of neurite length

Neurite length was measured using the Metamorph imaging system (Universal Imaging Co.). DRG grown on PLLA filaments with different treatments were immunolabeled with neurofilament antibody as described above. The images were captured, and the neurite length in each direction was determined by measuring the distance from the distal edge of growth to the DRG at the center, for each filament. The measurements were done on all filaments showing growth and values (pixel number) were converted to millimeters (mm).

RESULTS

In initial experiments, we observed poor attachment of DRG to uncoated PLLA filaments, indicating that these filaments, alone, are poor substrates for neuronal attachment and growth. To enhance attachment and neurite growth, PLLA filaments were pretreated with PLL, laminin, or PLL and laminin. To determine whether laminin bound to uncoated filaments and whether the amount bound could be increased by pretreating the filaments with PLL, we quantitated the amount of laminin bound to either uncoated or PLL-pretreated filaments using ELISA. The results showed that laminin bound PLLA filaments at a concentration of 0.8 ± 0.07 ng/mm,[2] whereas precoating the filaments with PLL did not increase the binding concentration of laminin (0.6 ± 0.06 ng/mm^2). To examine the stability of laminin bound to PLLA filaments and PLL-precoated filaments, the filaments were incubated at 37°C in culture medium and measured for the amount of laminin remaining bound daily, over an 8-day period. The measurements showed no change in the overall amount of laminin bound to the filaments over this time period (Fig. 1). In addition, no difference in laminin stability was observed between laminin-coated filaments and PLL + laminin-coated filaments.

Coating the filaments with laminin greatly enhanced the attachment of DRG and subsequent neurite growth compared with either uncoated or PLL-coated PLLA filaments. In these experiments, greater than 60% of all DRG attached and extended neurites on the laminin-coated filaments. In contrast, uncoated or PLL-coated filaments showed only 18 and 28% attachment of DRG, respectively, and poor neurite outgrowth. In all successful instances, DRG attached to two or more filaments, with neurites extending bidirectionally onto the attached filaments (Fig. 2). Occasionally, neurites were observed to jump and grow on filaments lying lateral to those on which the DRG was attached [Fig. 3(B)]. In these treatment groups, the majority of neurite outgrowth was unfasciculated and migrated in a linear direction along the longitudinal
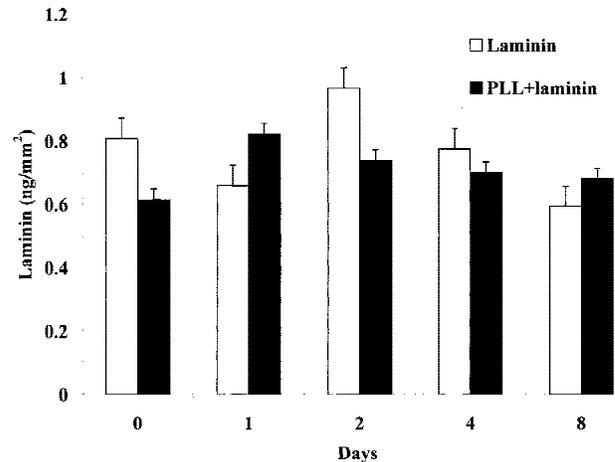

Figure 1. PLLA filaments coated with laminin (open bars) or PLL + laminin (solid bars) were incubated in DMEM + 10% FCS at 37°C for different time periods (0 to 8 days) to examine the stability of the bound laminin. The amount of laminin remaining bound was measured using laminin antibodies by ELISA. There was no significant difference in the amount of laminin remaining bound to either PLLA ($p = 0.83$) or PLL-precoated ($p = 0.96$) filaments over an 8-day period as evaluated by single factor ANOVA. Data are shown as means $\pm$ SEM ($n = 8$) of triplicate experiments.

axis of the filament (Fig. 2). Typically, neurite outgrowth over laminin-coated filaments was about 3 times that observed over uncoated or PLL-coated (control) filaments (Figs. 2 and 4). These results demonstrate the potential for laminin-coated filaments to guide axonal growth in a linear manner across a lesion gap in a nerve.

Robust Schwann cell migration was observed over the filaments in all treatment groups, as determined by immunocytochemical staining for p75 [Fig. 5(C)]. Schwann cell migration closely paralleled the extent of growth observed for neurites, in which a 3-fold increase in distance migrated was observed for laminin-coated filaments when compared with nonlaminin-coated filaments (Fig. 2, arrows). To further examine the interaction of Schwann cells and neurite growth cones, we initially attempted to double-label with antibodies against p75 and neurofilament; however, even unstained filaments showed very high fluorescent background. Alternatively, we chose to examine this association using scanning electron microscopy. For these studies, 3-day-old cultures were used to examine the relationship between growing neurites and Schwann cells. Even at this 3-day period, neurite outgrowth over laminin-coated filaments was extensive and much longer than that observed over PLL-coated filaments (Fig. 3). In several samples, neurites were observed to jump from one filament across to the neighboring filaments [Fig. 3(B)]. At the leading edge of growth on laminin-coated filaments, Schwann cells trailed the advancing neurites [Fig. 3(D)]. However, the leading edge of growth on PLL-coated filaments

Figure 2. Photomicrographs showing neurite growth on uncoated (A and D), PLL-coated (B and E), and PLL + laminin-coated (C and F) filaments. DRG growing on various filaments were immunostained with neurofilament antibodies. A–C represents the neurite outgrowth in the presence of Schwann cells and D–F, in the absence of Schwann cells. Striking differences in the extent of neurite growth were observed between various treatments (arrows mark the distal edge of growth). Laminin-coated filaments induced the longest neurites both in the presence and absence of Schwann cells compared with uncoated and PLL-coated filaments. Neurites fasciculated in the absence of Schwann cells on uncoated and PLL-coated filaments, whereas they remained mainly unfasciculated on laminin-coated filaments both in the presence and absence of Schwann cells. Original magnification ×50.

consisted of both migrating Schwann cells (arrowheads) and neurites (arrows), in which neurite growth did not appear to extend beyond the front of the Schwann cells [Fig. 3(C)]. These results indicate that the neurite growth is dependent on the rate of Schwann cell migration on PLL-coated filaments but independent of Schwann cell migration on laminin-coated filaments.

To determine the overall contribution Schwann cells

have on neurite outgrowth in this paradigm, we examined neurite growth in the presence and absence of Schwann cells. To eliminate Schwann cells, we photolyzed dividing cells after the attachment of DRG. In the absence of Schwann cells, the extent of neurite growth was reduced in all treatment groups (compare Fig. 2 A–C with D–F). Neurite growth over laminin-coated filaments showed the least apparent decrease, far exceeding the growth over uncoated or PLL-coated filaments, even in the presence of Schwann cells. In the absence of Schwann cells, neurites were observed to consistently form large fascicles on either uncoated or PLL-coated filaments [Fig. 2(D,E)], while remaining mostly unfasciculated on laminin-coated filaments [Fig. 2(F)]. Although laminin appeared to be sufficient to promote long distance growth in the absence of Schwann cells, their presence further increased the extent of neurite growth.

The results from the immunocytochemistry showed differences in the extent of neurite growth between various treatments. These differences in neurite growth were quantified by measuring the distance from the DRG to the distal edge of neurite growth after staining the neurites with neurofilament antibodies. These results demonstrate that laminin-coated filaments promote longer neurites when compared with uncoated and/or PLL-coated filaments (Fig. 4). The extent of growth on laminin-coated filaments averaged 5.8 ± 0.2 mm per filament in each direction from the DRG. This is 2.9 times higher than on uncoated (2 ± 0.2 mm) and 2.6 times higher than on PLL-coated (2.2 ± 0.3 mm) filaments. The loss of Schwann cells caused a drastic reduction in the neurite length on uncoated and PLL-coated filaments. Neurite length was reduced to 0.9 ± 0.1 mm on uncoated, 0.8 ± 0.1 mm on PLL-coated, and 3.9 ± 0.2 mm on laminin-coated filaments causing 55, 64, and 33% decrease, respectively. Although there was a significant decrease in the neurite length on laminin-coated filaments, it was comparatively less than on uncoated and PLL-coated filaments.

DISCUSSION

The primary goal of this study was to test whether the design of novel PLLA filaments would support the growth of Schwann cells and neurites from DRG explants. Initial results showed that PLLA filaments by themselves are poor substrates for DRG attachment and neurite growth; however, laminin greatly increased the attachment and neurite outgrowth while filaments directed the growth along their longitudinal axis. Laminin bound equally well to PLL-coated and to uncoated filaments, as measured by ELISA using laminin antibodies. Although it has been previously
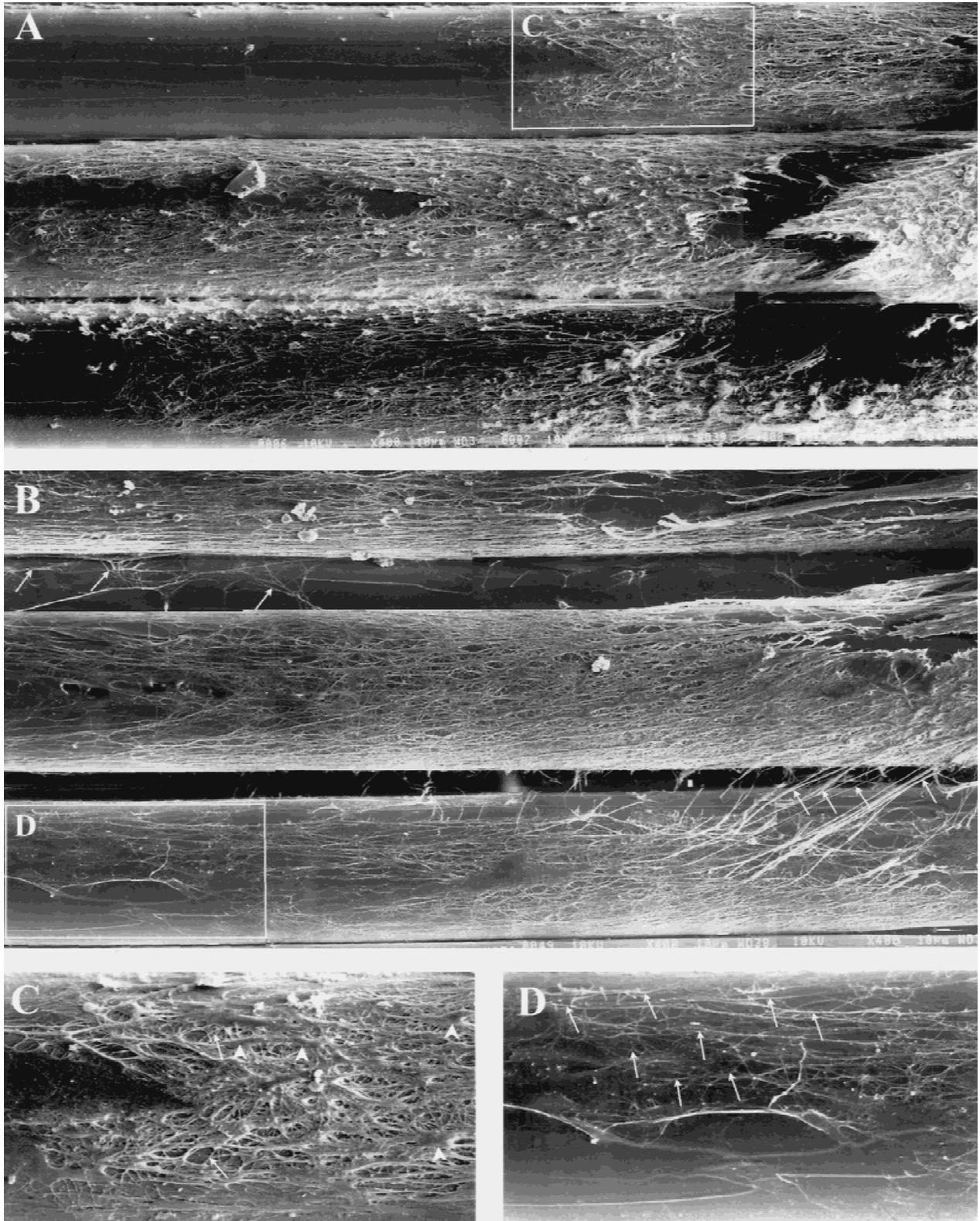
Figure 3. Montages illustrating the scanning electron microscopic images of DRG grown for 3 days on PLL-coated (A) and laminin-coated filaments (B). Extensive neurite growth was observed on laminin-coated filaments compared with PLL-coated filaments. Many neurites were found to jump across from one filament to the neighboring filament (arrows) on laminin-coated filaments. Boxes C and D show the leading edges of growth on PLL-coated and laminin-coated filaments, respectively. The higher magnification images of these fields show that the leading edges of growth on laminin-coated filaments (D) consisted of mainly neurites (arrows); no Schwann cells were apparent. The leading edge of growth on PLL-coated filaments (C) consisted of both neurites (arrows) and Schwann cells (arrowheads). A and B, original magnification ×150; C and D, original magnification ×300.
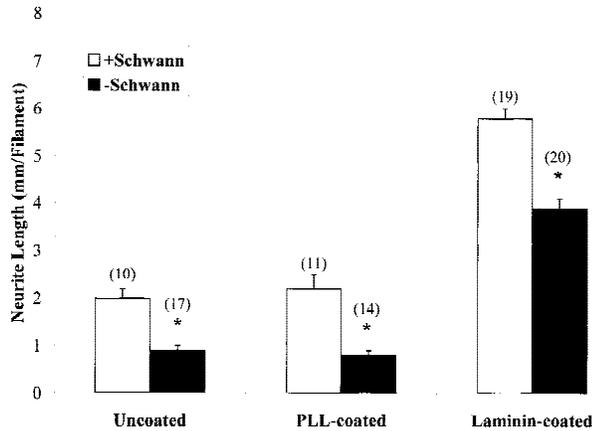
Figure 4. Bar graph showing the neurite length measurements obtained on PLLA filaments with various treatments. Significant differences in neurite length were observed between various treatments with Schwann cells (open bars) and without Schwann cells (solid bars). The neurite length was significantly higher in treatment groups with Schwann cells compared with groups without Schwann cells ($p < 0.001$). Laminin-coated filaments from both groups induced significantly longer neurites compared with uncoated and PLL-coated filaments ($p < 0.001$). Statistical significance was evaluated by student's t test. Data are shown as means ± SEM. The n values are shown in the parenthesis above the data point in the graph.

reported that precoating tissue culture plastic with either PLL or poly-L-ornithine greatly improves laminin binding,[36,37] we did not find significant changes in the amount of laminin binding to uncoated and PLL-precoated filaments ($p = 0.64$). Although laminin binding is independent of PLL precoating, a relatively high concentration adsorbed onto the surface of the filaments, approximately 0.8 ± 0.06 ng/mm^2. This concentration is almost equivalent to that observed to bind to tissue culture plastic, <1 ng/mm^2, but less than that observed after precoating the plastic with poly-L-ornithine, 3 ng/mm.[2,36] In previous studies, 2.5 ng of laminin in a 6-mm diameter culture well was enough to achieve 50% maximal neurite outgrowth.[38] If all the laminin bound to the well, the maximal concentration would be 0.09 ng/mm^2. Laminin binding to PLLA filaments in our study is approximately 8 times higher than the 50% maximal required to elicit neurite growth on poly-L-ornithine–laminin. In addition, bound laminin remained stable over 8 days in culture medium at 37°C, indicating that laminin interacts with PLLA with sufficient strength and that it does not diffuse out into liquid medium over time. Therefore, PLLA filaments can simply be coated with laminin without the use of crosslinking agents to secure to the surface; however, crosslinking agents will be required to achieve surface laminin concentrations higher than 1ng/mm^2.

The nature of binding and stability of laminin on the filament was observed to enhance neurite growth
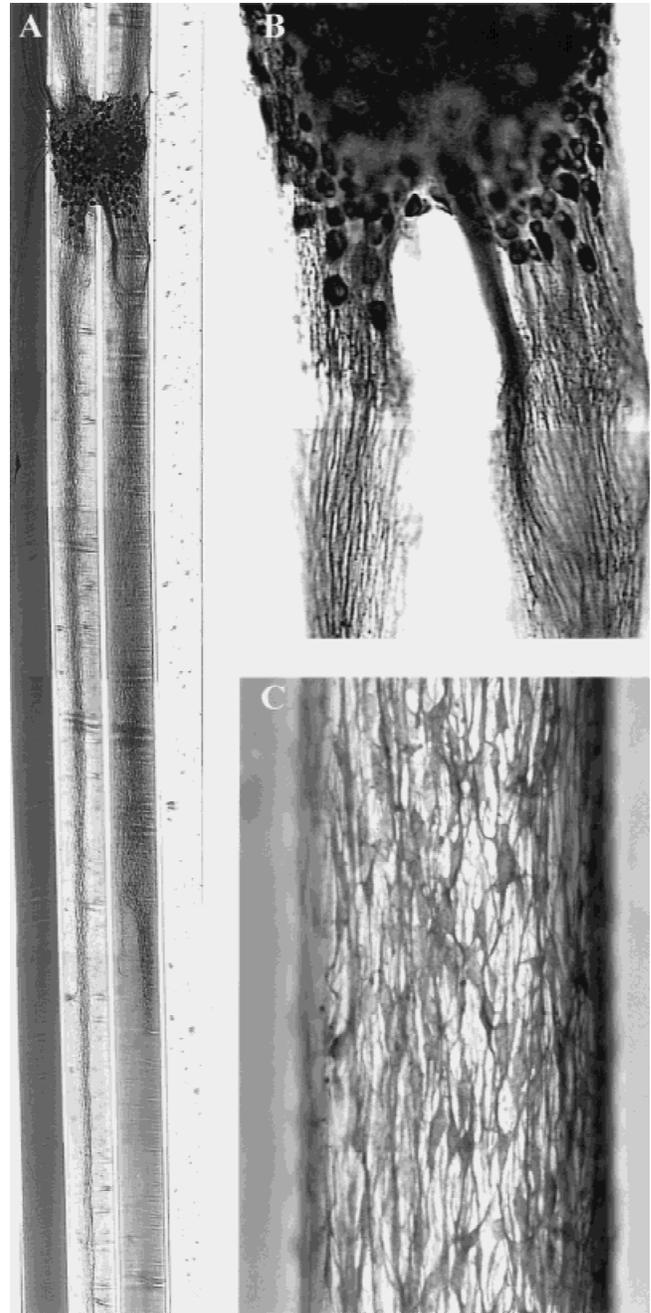

Figure 5. Micrograph showing the directional outgrowth of neurites on laminin-coated filaments. Many neurites emerge out of DRG (B) and grow longitudinally along the filaments (A). These neurites were not fasciculated and migrated unidirectionally toward the opposite end. Schwann cells immunostained with p75 antibodies were found to grow along the laminin-coated filaments (C). A, original magnification ×50; B and C, original magnification ×240.

more than 2.5 times than that of uncoated or PLL-coated (control) filaments. Neurite growth on laminin also appeared less fasciculated than control filaments. In the absence of Schwann cells, neurite outgrowth on laminin-coated filaments was still significantly greater than that observed on control filaments but less than

that observed on laminin-coated filaments with Schwann cells. At the farthest distance from the DRG, and only on laminin-coated filaments could the leading group of growth cones be observed growing ahead of the trailing Schwann cells. On these filaments, neurite growth seemed to be independent of Schwann cells and more dependent on the presence of laminin. Photolysis of Schwann cells, however, reduced the neurite length slightly more than 30%, indicating that Schwann cells still can enhance the growth of neurites, even on a laminin substrate. The enhancement of neurite outgrowth in the presence of Schwann cells may be due to their ability to express neurotrophins, neurite promoting factors[39,40] or heparan-sulfate proteoglycan, which can bind to laminin and further enhance neurite growth.[41,42]

The main objective in the design of these filaments was to provide a substrate that would direct axonal growth longitudinally across a lesion. Typically, neurite outgrowth from DRG explants in tissue culture is oriented in a radial pattern.[43] To force neurites to grow along a longitudinal pathway, we fabricated PLLA polymer into thin filaments. PLLA was chosen as the polymer of choice to fabricate filaments because of added advantages over the other bioresorbable polymers. We believe that the additional strength provided by PLLA permits the filaments to be drawn very fine, which in turn increases the surface area and hence contact guidance. The reduced polymer mass reduces the degradation burden and fosters the buffering of the protons released on degradation, thereby reducing the inflammation potential of the implant. An earlier report has indicated the possible role of increased acidity in inducing inflammatory reaction to the polymer implants.[44] We did not observe significant change in the pH when PLLA filaments were incubated in culture medium at 37°C for 8 days with or without DRG cultures (data not shown).

These filaments restrict the lateral migration of growth and consolidate growth into parallel arrays of neurites following the long axis of the filament. Essentially, each filament orients the growth in a manner similar to individual fascicles and is especially suited for guiding the axons toward the target site. This is important for the reestablishment of anatomical organization after injury, particularly after peripheral nerve injury, where the disorientation of growing axons hinders the functional recovery. Under such conditions, if the nerve is severed so that a gap persists, regenerating axons lose their orientation and can either enter inappropriate endoneural tubes in the distal stump or form neuromas.[1,2] However, if, after injury, the peripheral nerve remains intact, or the ends can be anastomosed, the continuity of the basal lamina tubes will act to guide regenerating axons, and functional restoration is quite good.[45] Similarly, the placement of nerve autograft, aligned matrix, or muscle basal lamina between the severed nerve stumps has been shown to enhance axon regeneration across a gap and preserve some of the organization.[46–48] Our results clearly demonstrate the suitability of the PLLA filaments to induce oriented axonal growth. By placing these filaments at the lesion site, it should be possible to orient the axonal growth longitudinally across the lesion site, thereby minimizing disoriented growth and perhaps, bringing about greater functional recovery.

Traumatic injury or invasive surgical procedures can cause large excisions of peripheral nerves that are separated by too large a distance for proper regeneration. To increase the prospects for axonal regeneration across a gap between nerve stumps, nerve ends have been placed into tubes of various materials. Successful regeneration within these tubes first requires the formation of a fibrin clot matrix within the chamber.[23,24] Schwann cells and fibroblasts then migrate toward the center of the fibrin matrix from both nerve ends to produce a cable that extends from one end to the other. Regenerating axons from the proximal nerve follow the growth of Schwann cells, being directed presumably by the chemotaxic gradient established within the chamber.[49] If the gap between the nerves is increased to a distance greater than 1 cm in the rat, cable formation is tenuous, and axon regeneration rarely occurs. Placement of PLLA filaments into the chamber of a tube could form a bridge that can organize the migration of non-neuronal cells as well as the growth of axons across the gap. Such a design, in addition to being bioresorbable, offers several advantages over most other models. The thin filaments have greater surface area for cellular interaction and migration, and the spaces between the filaments form channels to allow the diffusion of growth factors. In preliminary studies, we have observed robust axonal regeneration with filaments placed in a 1-cm gap between severed ends of the rat sciatic nerve. These regenerating axons were observed to form several well-defined fascicles.

These filaments can further be made microporous and be loaded with neurotrophic factors, which are released as they degrade and thus acting as a neurotrophin reservoir. This should greatly enhance the axonal growth rate. Another alternative is to seed activated Schwann cells onto the filaments. Longitudinal migration of Schwann cells along the filaments may act similar to bands of Bungner, further aiding regeneration *in vivo*. In conclusion, a neural transplant composed of longitudinally aligned, laminin-coated PLLA filaments, loaded with neurotrophins perhaps may open up new perspectives for repair and improve functional recovery after injury in the central nervous system or over larger gaps in the PNS.

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