Treatment of a Segmental Nerve Defect in the Rat with Use of Bioabsorbable Synthetic Nerve Conduits: A Comparison of Commercially Available Conduits

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Treatment of a Segmental Nerve Defect in the Rat with Use of Bioabsorbable Synthetic Nerve Conduits: A Comparison of Commercially Available Conduits

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Background: The use of biodegradable synthetic nerve conduits for the reconstruction of segmental nerve defects has been extensively reported in both animal and human studies, with a majority of studies evaluating sensory nerve recovery. However, few studies have compared these nerve conduits for functional motor recovery. The purpose of this study was to compare three commercially available, synthetic, bioabsorbable nerve conduits and autograft with respect to compound muscle action potentials, maximum isometric tetanic force, wet muscle weight, and nerve histomorphometry.

Methods: Eighty Lewis rats were divided into four groups according to the type of repair of a 10-mm excision of the sciatic nerve: group I had a reversed autograft; group II, a poly-DL-lactide-ε-caprolactone conduit; group III, a type-I collagen conduit; and group IV, a polyglycolic acid conduit. All results were compared with the contralateral side. At twelve weeks, the rats underwent bilateral measurements of the compound muscle action potentials of the tibialis anterior and flexor digiti quinti brevis muscles, isometric tetanic force and muscle weight of the tibialis anterior, and peroneal nerve histomorphometry.

Results: At twelve weeks, no difference in the percentage of recovery between the autograft and the poly-DL-lactide-ε-caprolactone conduit was observed with respect to compound muscle action potentials, isometric muscle force, muscle weight, and axon count measurements. The poly-DL-lactide-ε-caprolactone and collagen conduits remained structurally stable at twelve weeks, while the polyglycolic acid conduits had completely collapsed. The polyglycolic acid conduit had the poorest results, with a recovery rate of 15% for compound muscle action potentials and 29% for muscle force.

Conclusions: The functional outcome in this rat model was similar for the autograft and the poly-DL-lactide-ε-caprolactone conduits when they were used to reconstruct a 10-mm sciatic nerve defect. Functional recovery following the use of the polyglycolic acid conduit was the poorest.

Clinical Relevance: Differences were demonstrated between commercially available conduits in this rat model. These results will allow surgeons to choose the optimal bioabsorbable synthetic conduit for human segmental nerve defect reconstruction.

Attaining adequate functional recovery following the repair of a segmental peripheral nerve defect has challenged surgeons. It has been long established that, in the repair of a segmental defect of a nerve, coaptation of the stumps under tension leads to very poor results[1-4]. In such cases, an interposition autologous nerve graft is considered the gold standard and has produced the best results[5]. Although autologous nerve graft has been considered the gold standard, its use is not without problems. The limited supply, associated donor-site morbidity, and a size mismatch with the injured

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nerve can occur[6]. The concept of a preformed tissue space created by a nerve conduit for nerve regeneration has been explored for nearly three decades[7-9]. Nerve conduits are theoretically advantageous compared with autografts in that they have an unlimited supply, have no donor-site morbidity, are not restricted by fascicular sizes, and provide the regenerating nerve fibers with the ability to seek their own path to their targets with a high degree of specificity through neurotropism[5,10,11].

Following nerve repair, the recovery of motor and sensory function requires the survival and regeneration of axons, synaptogenesis, and recovery of the end organ[12,13]. Although much has been reported with respect to sensory nerve recovery after conduit reconstruction[14-16], only case reports have described motor nerve recovery after repair with use of a conduit[17-21]. Motor nerve reconstruction is fundamentally different from sensory nerve reconstruction as there are time-dependent degradation and ultrastructural changes of the muscle and irreversible changes that occur at the motor end plate[22,23]. Few studies have compared motor outcomes after reconstruction of segmental nerve defects with conduits in humans[16], and, although recovery has been explored in animals, evaluation has been primarily passive or indirect with use of methods of measurement such as compound muscle action potentials and walking track analysis.

Currently, three synthetic bioabsorbable nerve conduits are approved for clinical use in peripheral and cranial nerve repair in the United States and Europe; these include the Neurolac tube (poly DL-lactide-ε-caprolactone; Ascension Orthopedics, Austin, Texas), NeuraGen tube (type-I collagen; Integra LifeSciences, Plainsboro, New Jersey), and Neurotube (polyglycolic acid; Synovis, Birmingham, Alabama)[24]. To date, no study, as far as we know, has compared the efficacy of these three types of material in motor nerve reconstruction. The purpose of the present study was to compare the three commercially available, bioabsorbable, synthetic nerve conduits and a reverse autograft in a rat model of a segmental sciatic nerve defect with respect to compound motor action potentials of the tibialis anterior, maximum isometric force measurement of the tibialis anterior, wet muscle weight, and nerve histomorphometry.

Materials and Methods

This study was approved by the institutional review board and Institutional Animal Care and Use Committee. A unilateral 10-mm sciatic nerve defect model in male Lewis rats (200 to 300 g) was used. Eighty rats were divided into four experimental groups: group I was repaired by a reverse segmental autologous graft obtained from the same defect, group II underwent repair with a 1.5-mm-diameter poly-DL-lactide-ε-caprolactone conduit (Neurolac; Ascension Orthopedics), group III received a 1.5-mm-diameter type-I collagen conduit (NeuraGen; Integra), and group IV was repaired with use of a 2.3-mm-diameter woven polyglycolic acid mesh conduit with corrugated walls (Neurotube; Synovis). Ideally, a 1.5-mm-diameter tube would have been used; however, the smallest diameter tube available from Synovis was the 2.3-mm-diameter tube. The nerve reconstructions were randomized into the four groups prior to the start of the study. To minimize side-to-side variability, odd-numbered rats received a repair on the left side and even-numbered rats received a repair on the right during the initial procedure. The contralateral side from the repair served as an intraspecimen control.

The initial procedure for all rats included nerve injury and repair as well as baseline bilateral measurements of the compound muscle action potential. The repair was allowed to heal during a twelve-week recovery period, during which the rats were given food and water ad libitum and were housed individually with a twelve-hour light-dark cycle. Following the twelve-week recovery period, a final procedure was performed and compound muscle action potentials, force, weight, and histomorphometry were evaluated. The experimenter was blinded to the randomization and type of repair for the final procedure when bilateral measurements of isometric tetanic muscle force and compound muscle action potentials of the tibialis anterior were performed. At the end of the final procedure, tissue was harvested for muscle weight and histomorphometric measurements.

One rat in group IV did not survive the twelve-week recovery period. During the final procedure, two rats from group II, one from group III, and two from group IV were not able to complete the isometric tetanic force test because of anesthesia complications.

Initial Procedure: Initial Measurements of Compound Muscle Action Potential and the Nerve Injury and Repair

All rats were anesthetized with an intraperitoneal injection of ten parts of ketamine (Ketaset; Fort Dodge Animal Health, Fort Dodge, Iowa) and one part of xylazine (VetTek, Blue Springs, Missouri) at a dosage of 1 mg/kg of body weight. To maintain anesthesia, subsequent doses of ketamine were given intramuscularly. Body temperature was maintained at 37°C with a heating pad, and 5 mL of lactated Ringer solution and 0.02 mL of trimethoprim-sulfadiazine (48%; Five Star Compounding Pharmacy, Clive, Iowa) were administered subcutaneously. Once the rats were anesthetized, sterile technique was maintained throughout the procedure. Initially, the sciatic trunk was exposed bilaterally through a dorsal approach at the posterolateral aspect of the thigh for electromyographic measurements. A miniature bipolar stimulating electrode (Harvard Apparatus, Holliston, Massachusetts) was clamped around the exposed sciatic nerve, and a ground electrode was placed in the surrounding musculature. Bipolar recording electrodes were placed in the tibialis anterior and the flexor digiti quinti brevis muscle, one near the motor end plate and the other distally. With use of a VikingQuest Portable electromyography system (Nicolet Biomedical, Madison, Wisconsin) and VikingQuest software on a personal computer, the compound muscle action potentials were measured in the tibialis anterior and flexor digiti quinti brevis muscles. Stimulation duration was 0.02 msec, and the minimum intensity needed to elicit a maximum compound muscle action potential signal was used.

Following bilateral measurements of the compound muscle action potentials, the control side was irrigated and closed with a muscle reapproximation with 4-0 absorbable

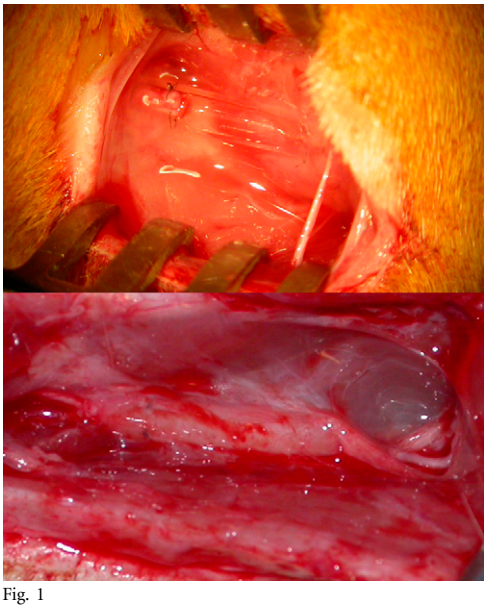

Fig. 1

Group-II (poly-DL-lactide-ε-caprolactone) repair at zero (top) and twelve weeks (bottom).

sutures and a skin closure with 4-0 nylon sutures. The experimental side was left open for subsequent nerve resection and repair. The time interval between the initial incision and skin closure of the control side for the electromyographic measurements typically lasted fifteen minutes. With the experimental side still exposed, the rats were then divided on the basis of the randomized group assignment.

Nerve Injury and Repair in Group I

The sciatic nerve of the experimental side was fully exposed by extending the skin incision approximately 2 cm from the midline slightly caudal and parallel to the femur to approximately 2 cm proximal to the knee joint. The nerve was exposed from the inferior margin of the piriformis to approximately 5 mm past the bifurcation of the peroneal and tibial branches. The sural branch was resected and discarded. A 10-mm segment of the sciatic nerve was excised 5 mm distal to the early tibial branch, proximal to the knee joint, by a sharp transection with a microsurgical scissor under an operating microscope (Zeiss OpMi 6; Carl Zeiss Surgical, Oberkochen, Germany). The excised sciatic nerve segment was then reversed and grafted between the proximal and distal nerve stumps with use of six 10-0 nylon epineurial interrupted sutures at each neurorrhaphy. The surrounding musculature was reapproximated over the repair with 4-0 absorbable sutures, and a skin closure was performed with 4-0 nylon sutures. Following skin closure, 0.05 mg of Buprenex (buprenorphine opioid analgesic; Reckitt Benckiser Pharmaceuticals, Richmond, Virginia) was administered subcutaneously. Postoperatively, the rats were kept warm with a heating pad until they were stable and were given 960 mg of acetaminophen (Q-pap; Qualitest Pharmaceuticals, Huntsville, Alabama) added once to the feed water. All rats were observed daily until the completion of the experiment.

Nerve Injury and Repair in Groups II, III, and IV

The approach and nerve segment resection was identical to that in group I. The 10-mm gap was then repaired with the appropriate nerve conduit (poly DL-lactide-ε-caprolactone, type-I collagen, or polyglycolic acid) and secured by two 8-0 nylon transverse epineurial loop sutures at each junction, as per the manufacturer's recommendation. The lumen was flushed with saline solution, and fibrin sealant (TISSEEL; Baxter, Westlake Village, California) was applied over the ends of the nerve conduit (Figs. 1, 2, and 3, top). Fibrin glue sealant was used to occlude the gap between the nerve and conduit to prevent dislodgment of the nerve ends and hematoma from entering the nerve conduits. Care was taken not to introduce any sealant into the lumen of the conduits between the nerve ends. Closure and postoperative treatment were identical to those in group I.

Final Procedure: Electromyographic and Force Measurement

Final Measurements of Compound Muscle Action Potential

At twelve weeks, the rats were anesthetized with an intraperitoneal injection of ten parts of ketamine and one part of xylazine at a dosage of 1 mL/kg of body weight. Subsequent doses of ketamine were administered intramuscularly to maintain anesthesia. Following anesthesia, 5 mL of lactated Ringer solution was administered subcutaneously. With use of the same dorsal approach as the initial procedure, the main sciatic trunk and the peroneal branch of the sciatic nerve were exposed. As in the measurements of compound muscle action potential in the initial procedure, ground and collecting electrodes were placed in the adjacent tissue, the tibialis anterior, and the flexor digiti quinti brevis. The bipolar stimulating electrode was placed proximal to the still visible repair (or the equivalent location for the control side) on the sciatic trunk, and compound muscle

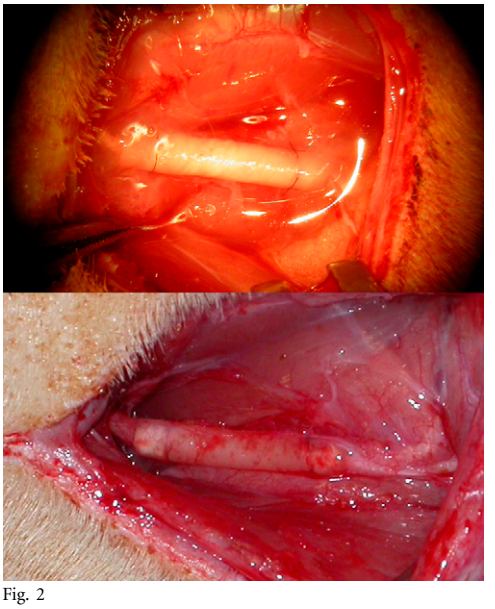

Fig. 2

Group-III (collagen) repair at zero (top) and twelve weeks (bottom).

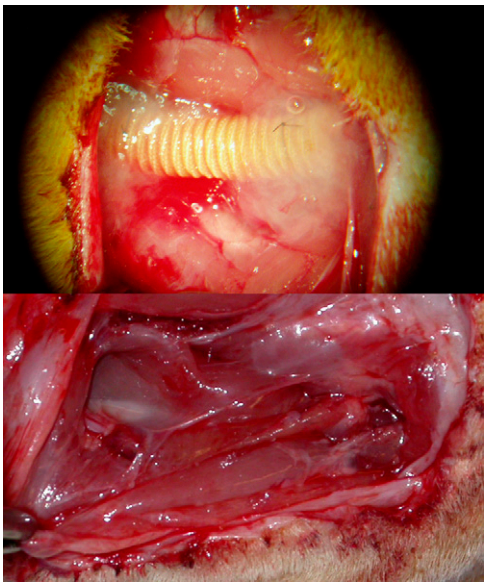

Fig. 3

Group-IV (polyglycolic acid) repair at zero (top) and twelve weeks (bottom). The polyglycolic acid tube has completely collapsed.

action potentials were measured as they were performed previously in the initial procedure. The skin was temporarily reapproximated with suture on completion of the compound muscle action potential testing until force measurements were conducted. Exposure and measurements were also repeated for the contralateral side.

Measurement of the Maximum Isometric Tetanic Force

Following the compound muscle action potential measurements, maximum isometric tetanic muscle force measurements were performed bilaterally as previously detailed by Shin et al.[25]. The tibialis anterior tendon was released at its insertion, and the muscle was freed from the surrounding tissue while preserving its neurovascular pedicle. The hind limb was secured to a testing board with Kirschner wires (DePuy Orthopaedics, Warsaw, Indiana), and the distal tibialis anterior tendon was attached to a force transducer (MDB-2.5; Transducer Techniques, Temecula, California) by a custom clamp. The tibialis anterior was positioned close to its original anatomical orientation. The force transducer signal was processed on a personal computer with use of LabVIEW (National Instruments, Austin, Texas). A bipolar stimulator (Grass SD9; Grass Instruments, Quincy, Massachusetts) was clamped around the peroneal branch with use of a miniature electrode (Harvard Apparatus) with the nerve kept in situ (Fig. 4). During force testing, body temperature was maintained at 37°C with a heating pad (K-module Model K-20; American Pharmaseal, Valencia, California), and the muscle was kept moist with a heated saline-solution drip (37°C). When the maximum isometric tetanic force of the tibialis anterior was measured[25], four parameters were identified and optimized: muscle length, stimulus intensity, pulse duration, and pulse frequency, in that order. After completion of the force testing, the skin was reapproximated with suture and the isometric tetanic force testing was repeated for the contralateral side.

Tissue Collection

Following the bilateral measurements of the maximum isometric tetanic force, the tibialis anterior muscles were carefully dissected from the surrounding tissues, removed from the body, and weighed. The rats were perfused with 60 mL of lactated Ringer solution, 120 mL of heparinized saline solution, and 150 mL of fixative (2% paraformaldehyde and 1% glutaraldehyde in 0.1 M phosphate buffer). A 5-mm segment of the peroneal branch of the sciatic nerve distal to the repair was dissected bilaterally. Collected tissue was stored in the fixative.

Histomorphometry

The nerve tissue samples were embedded in epoxy resin, cut into 1-μm cross sections and stained with toluidine blue. Histomorphometric analysis was performed by Digital Cell Imaging Labs (Edegem, Belgium) for axon counts, nerve area, mean internal nerve fiber area, and mean myelin thickness. Nerve area

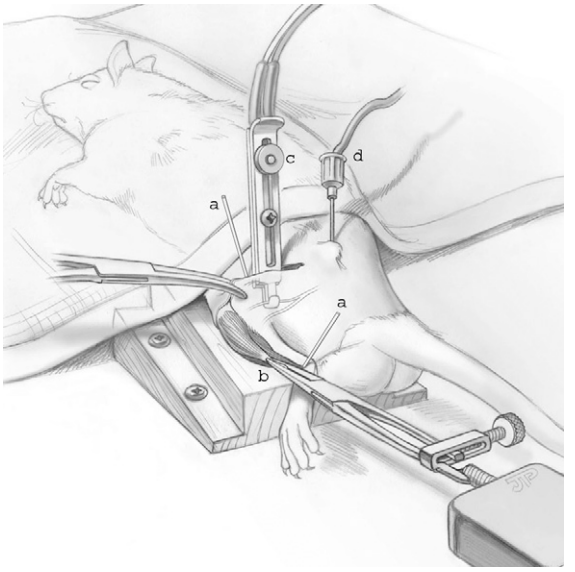

Fig. 4

Schematic drawing of the experimental setup for isometric tetanic force testing. The rat is prone with the femur and ankle attached to the testing block with two Kirschner wires (a). The force transducer is attached to the tibialis anterior muscle by a custom clamp on the distal tibialis anterior tendon (b). A bipolar electrode is attached to the peroneal branch of the sciatic nerve (c), and the rat is electrically grounded (d). (Copyrighted and used with permission of the Mayo Foundation for Medical Education and Research; all rights reserved. Reprinted from: Shin RH, Vathana T, Giessler GA, Friedrich PF, Bishop AT, Shin AY. Isometric tetanic force measurement methods of the tibialis anterior in the rat. Microsurgery. 2008;28:452-7.)

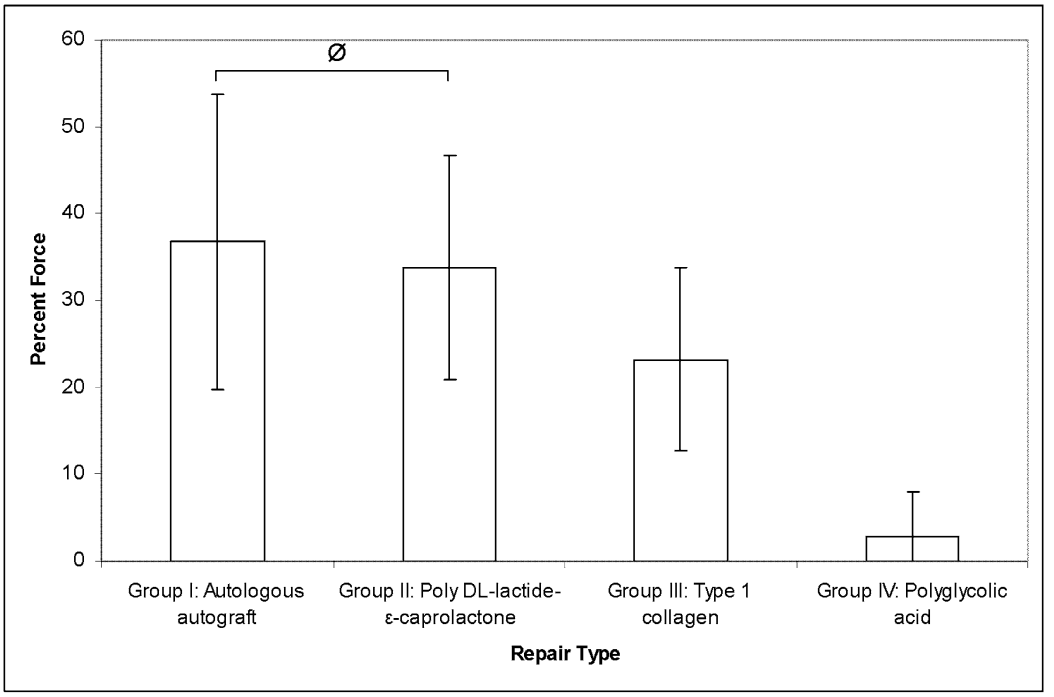

Fig. 5

Maximum isometric tetanic force of the tibialis anterior for the four test groups. The values are expressed as a percentage of the contralateral, control side and are given as the mean and the standard deviation. Ø indicates no significant difference at $p < 0.05$, with all other comparisons being significant.

was measured as the area encircling the outermost fibers of the peroneal nerve. Internal fiber area was determined to be the internal axon area or area within the circumferential myelin sheath. Myelin thickness was calculated as the distance between the outer and inner sheath circumferences.

Statistical Analysis

Prior to commencement of the study, a power analysis was performed to determine the number of rats in each group on the basis of previous studies of isometric motor force[25]. Assuming similar variability would be observed in this study, a sample of fifteen rats in each of the four groups would provide 80% power to detect a difference in mean muscle force of 11% between any two of the four study groups (alpha = 0.05, two-sided test). In order to guard against potential attrition and to overpower the study, the per-group sample size was increased to twenty, yielding a total sample of eighty rats. Isometric motor force was to be the primary outcome; thus, power calculations were not performed for compound muscle action potential or histomorphometric comparisons.

The repeatability and consistency of the maximum isometric tetanic force measurements had already been established[25]. In order to determine the repeatability and consistency of compound muscle action potential measurements, the bilateral measurements at zero weeks were assessed by comparing the 95% confidence interval with an equivalence interval of ±15%. Equivalence was determined to be acceptable when the 95% confidence interval fell within the ±15% equivalence interval.

Groups I through IV (autologous graft, poly-DL-lactide-ε-caprolactone, type-I collagen, and polyglycolic acid conduits, respectively) were compared with respect to the maximum isometric tetanic force of the tibialis anterior, compound muscle action potentials of the tibialis anterior and flexor digiti quinti brevis, tibialis anterior muscle weight, and nerve histomorphometry (axon count, nerve area, fiber area, and myelin thickness). The Kruskal-Wallis test was used to determine differences in tetanic force and compound muscle action potentials, while the Wilcoxon signed-rank test was used for individual two-group comparisons. Differences in muscle weight and histomorphometric analysis were determined with use of a one-way analysis of variance. Further analysis to determine differences between groups was performed with use of the adjusted Holm t test. Zero recovery values were included in the calculation of the mean for muscle force and compound muscle action potentials, but not for axon counts. Recovery rate was defined as the percentage of animals per group that yielded a nonzero force or electromyographic signal from the muscle tested on the repair side:

$$\text{Recovery Rate} = \left(\frac{\text{Number of animals with repair side force/signal} > 0}{\text{Total animals completing force/electromyographic testing}} \right) \times 100$$

Unadjusted p values of <0.05 were considered significant, and all values were reported as the mean and the standard deviation.

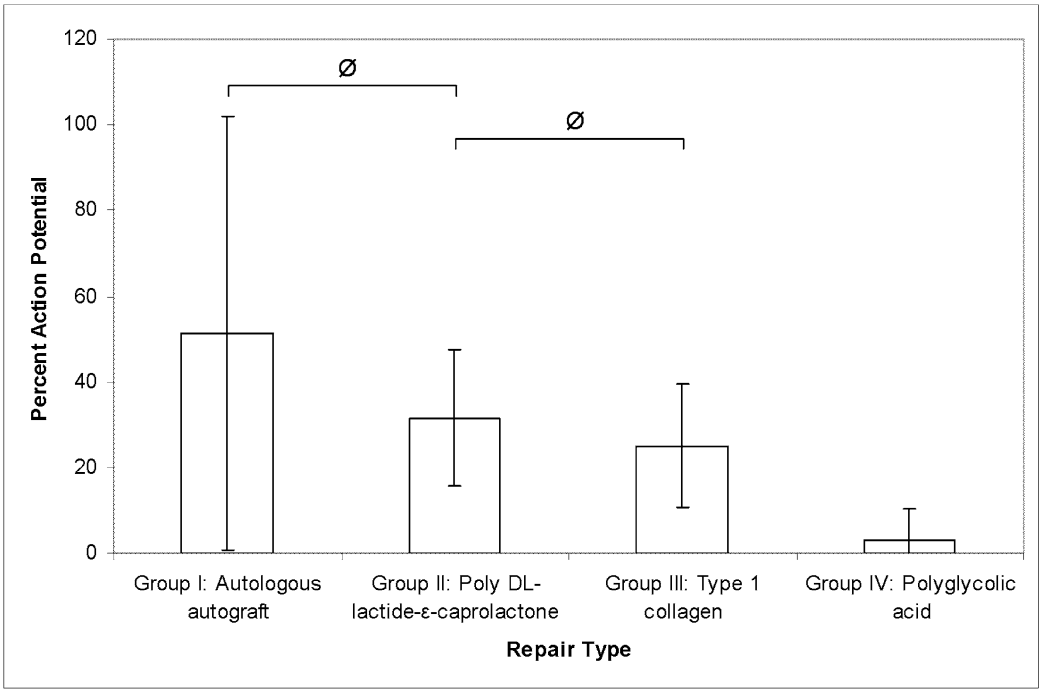

Fig. 6

Compound muscle action potentials of the tibialis anterior for the four test groups at twelve weeks. The values are expressed as a percentage of the contralateral, control side and are given as the mean and the standard deviation. Ø indicates no significant difference at $p < 0.05$, with all other comparisons being significant.

Source of Funding

This study was funded by the Mayo Foundation Research Grant (CR-20). All nerve conduits were donated by Integra LifeSciences, Ascension Orthopedics, and Synovis.

Results

The mean body weight of the rats at twelve weeks was 392 ± 28 g, with no significant difference found among the groups ($p = 0.71$). Of the eighty rats, one rat from group IV did not survive during the twelve-week recovery period. Although degradation was observed in all conduits, the implants were still present at twelve weeks in all of the surviving rats (Figs. 1, 2, and 3, bottom). The polyglycolic acid conduits in group IV did not retain their tubular structure and had completely collapsed at twelve weeks. One blood clot was present at the nerve stump of a failed repair from group II, but no blood clots or substantial obstructions were observed in the lumens of any of the other repairs.

Maximum Isometric Tetanic Force Measurement

The tibialis anterior isometric tetanic force recovery was 37% ± 17% for group I (autograft), 34% ± 13% for group II (poly DL-lactide-ε-caprolactone), 23% ± 11% for group III (type-I collagen), and 3% ± 5% for group IV (polyglycolic acid) (Fig. 5) at twelve weeks. Significant differences in muscle force were found between all groups except between groups I (autograft) and II (poly DL-lactide-ε-caprolactone) ($p = 0.71$). Groups I and III showed a 100% rate of functional recovery (all twenty and all nineteen repairs, respectively) of the tibialis anterior muscle, whereas group II had a 94% rate of recovery (seventeen of eighteen repairs) and group IV had the lowest rate of recovery at 29% (five of seventeen repairs).

Compound Muscle Action Potential Measurements

During the initial procedure (zero weeks), prior to injury and repair, the mean compound muscle action potential of the repair sides from all groups as a percentage of the contralateral, control sides for the tibialis anterior was 100.1% ± 18.8%, with a 95% confidence interval around the mean of 104.8% to 95.2%. For the flexor digiti quinti brevis, the mean was 102.5% ± 37.1%, with a 95% confidence interval around the mean of 112.0% to 93.2%. Both of these intervals fall within the ±15% equivalence interval.

At twelve weeks, the repair-side tibialis anterior stimulated by the sciatic nerve proximal to the repair exhibited a compound muscle action potential recovery of 51% ± 51% for group I, 32% ± 16% for group II, 25% ± 14% for group III, and 3% ± 7% for group IV (Fig. 6). Significant differences were found between all groups except between groups I and II (autograft and poly DL-lactide-ε-caprolactone, respectively) ($p = 0.46$) and groups II and III (poly DL-lactide-ε-caprolactone and type-I collagen) ($p = 0.21$). At twelve weeks, the compound muscle action potential for the flexor digiti quinti brevis muscle as a percentage of the contralateral side was 10% ± 12% for group I, 10% ± 11% for group II, 3% ± 4% for group III, and 0% for group IV (Fig. 7). A significant difference was

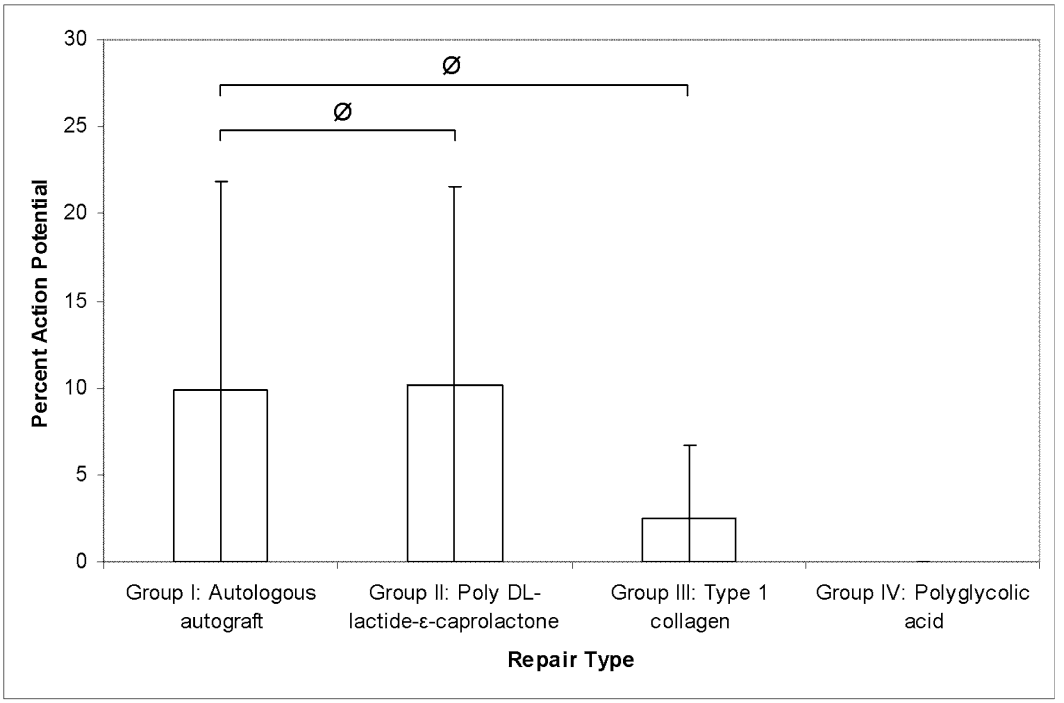

Fig. 7

Compound muscle action potentials of the flexor digiti quinti brevis for the four test groups at twelve weeks. The values are expressed as a percentage of the contralateral, control side and are given as the mean and the standard deviation. Ø indicates no significant difference at $p < 0.05$, with all other comparisons being significant.

found between all groups except groups I and II ($p = 0.83$) and groups I and III ($p = 0.11$). In group I, the rate of recovery of compound muscle action potential was 100% (fifteen of fifteen repairs) for the tibialis anterior and 53% (eight repairs) for the flexor digiti quinti brevis muscles. In group II, the rate of recovery of compound muscle action potential was 93% (fourteen of fifteen repairs) for the tibialis anterior muscles and 73% (eleven repairs) for the flexor digiti quinti brevis muscles. Group III had a 100% and 33% rate of recovery of compound muscle action potential for the tibialis anterior (all fifteen repairs) and flexor digiti quinti brevis muscles (five of fifteen), respectively. In group IV, the rate of recovery of compound muscle action potential was 15% (two of thirteen) for the tibialis anterior and 0% (none) for the flexor digiti quinti brevis.

Muscle Weight

Final weight for the tibialis anterior muscle was 58% ± 7%, 54% ± 10%, 49% ± 9%, and 19% ± 8% for groups I through IV, respectively. Significant differences in muscle weight were found between all groups except between groups I and II ($p = 0.11$) and groups II and III ($p = 0.10$).

Histomorphometry

Axon counts normalized to the contralateral control side for the peroneal nerve were 118% ± 24% for group I, 112% ± 23% for group II, 86% ± 15% for group III, and 47% ± 16% for group IV. Significant differences were found between all groups except between groups I and II ($p = 0.65$). No difference was found among the groups with respect to nerve area, mean internal nerve fiber area, or mean myelin thickness. A summary of all of the results is presented in Table I.

Discussion

The use of nerve conduits for motor nerve reconstruction requires greater consideration than sensory recovery because of the important regenerative limitations of motor function. Indeed, clinically it has been observed that motor recovery is inferior to sensory recovery following nonsegmental nerve repair in children[26]. A repaired transected motor nerve, delayed for fifty-six days in the rat, fails to recover[27]. Intervention must be timely and optimal.

As discussed previously, nerve autograft has resulted in the best results and is considered the gold standard, yet is hampered by donor-site morbidity, limited supply, and potential fascicular mismatch. The bioabsorbable nerve conduit is a potentially effective alternative to the autograft. However, most clinical studies evaluating nerve recovery following tubulization repair have focused primarily on sensory function[6,16,28-30], which is fundamentally different from motor recovery. Clinical case reports and series on motor nerve repair with use of synthetic conduits have been conducted but are limited in size and number and make no comparison between different conduit materials[18-21]. Meyer et al. compared end-to-end epineurial repair and a nondegradable silicone nerve conduit in the rat with respect to isometric contractile properties and nerve

TABLE I Summary of Results by Test for All Groups

	Group I	Group II	Group III	Group IV
Type of conduit*	Autograft	Neurolac Tube	NeuraGen Tube	Neurotube
Material	Autologous	Poly DL-lactide-ε-caprolactone	Type-I collagen	Polyglycolic acid
Rat initial weight† *(%)*	260 ± 14	256 ± 13	259 ± 14	251 ± 15
Rat final weight† *(%)*	393 ± 32	397 ± 36	390 ± 20	386 ± 23
Isometric tetanic force (normalized to contralateral side)				
Anterior isometric tetanic force recovery of tibialis anterior† *(%)*	37 ± 17	34 ± 13	23 ± 11	3 ± 5
Recovery rate of tibialis anterior‡	100 (20/20)	94 (17/18)	100 (19/19)	29 (4/17)
Compound muscle action potentials (normalized to contralateral side)				
Recovery of tibialis anterior† *(%)*	51 ± 51	32 ± 16	25 ± 14	3 ± 7
Recovery rate of tibialis anterior‡	100 (15/15)	93 (14/15)	100 (15/15)	15 (2/13)
Recovery of flexor digiti quinti brevis† *(%)*	10 ± 12	10 ± 11	3 ± 4	0
Recovery rate of flexor digiti quinti brevis‡	53 (8/15)	73 (11/15)	33 (5/15)	0 (0/15)
Weight (normalized to contralateral side)				
Muscle weight of tibialis anterior† *(%)*	58 ± 7	54 ± 10	49 ± 9	19 ± 8
No. tested	20	20	20	19
Histomorphometry (normalized to contralateral side)† *(%)*				
Peroneal axon count	118 ± 24	112 ± 23	86 ± 15	47 ± 16
Peroneal nerve area	89 ± 17	88 ± 20	74 ± 13	58 ± 11
Peroneal nerve fiber area	39 ± 4	34 ± 5	41 ± 19	35 ± 2
Peroneal myelin thickness	87 ± 9	84 ± 8	90 ± 5	76 ± 7

*Neurolac tube is manufactured by Ascension Orthopedics (Austin, Texas); NeuraGen tube, by Integra LifeSciences (Plainsboro, New Jersey); and Neurotube, by Synovis (Birmingham, Alabama). †The values are given as the mean and the standard deviation. ‡The values are given as the percentage, with the number of repairs that had recovered divided by the total number with complete data in parentheses.

conduction studies[31]. While recovery was better in the epineurial repair group than in the conduit repair group at eight weeks, no difference was found between the groups at sixteen or thirty-two weeks. The authors concluded that the use of a nerve guide conduit was a potential alternative to epineurial repair. Zhao et al. measured the tetanic tension at twelve weeks following various nerve repair methods including epineurial repair and silicone tubulization in the rat[11]. While epineurial repair produced superior force recovery, tubulization resulted in a higher degree of specificity for reinnervation[11]. Other studies have demonstrated that a conduit fabricated from type-I collagen was as effective as an autograft with respect to electrophysiologic and histomorphometric properties of the target muscles in primates and rats; however, no direct measure of motor force recovery was performed[32,33].

The use of compound muscle action potentials to quantify motor recovery has been known to have high variability and is an indirect measurement of motor function[34-36]. Other means of quantifying motor recovery that have been used include walking track analysis, which is also another indirect means of measuring motor recovery. Maximum isometric tetanic force acquired with optimization of variables has been shown to be an effective direct measure of functional recovery with high side-to-side consistency in the rat model and was chosen as the primary measure of motor outcomes in this study[25].

As we know of no comparison of commercially available bioabsorbable conduits in the three different available materials with use of a direct assessment of motor recovery, we sought to compare them in a rat sciatic nerve. Our results showed no significant difference between the poly-DL-lactide-ε-caprolactone conduit (group-II) and autograft (group-I) repairs in all assessments. The type-I-collagen conduit (group-III) repairs had inferior results compared with the poly-DL-lactide-ε-caprolactone conduit (group-II) repairs except with regard to tibialis anterior compound muscle action potentials and muscle weight measurements. The polyglycolic acid conduit (group-IV) repairs were significantly worse than other repairs in all assessments.

Waitayawinyu et al. compared the polyglycolic acid and the type-I collagen conduits and a nonrotated autograft in the rat model[37]. Following a fifteen-week regeneration period, the polyglycolic acid conduit yielded significantly worse results than the autograft and the type-I collagen conduit in isometric muscle contraction, axon counts, and muscle weights. Our study is in general agreement with this observation. Although

the type-I-collagen conduit repair was significantly less effective than the autograft repair except for the flexor digiti quinti brevis compound muscle action potentials, they were both more effective than the polyglycolic acid conduit in all parameters.

Compound muscle action potentials of the tibialis anterior were also observed by Valero-Cabré et al. in the examination of the repair of an 8-mm nerve gap with an autograft, a poly-DL-lactide-ε-caprolactone conduit, and a silicone conduit[38]. At ninety days, a comparison of the repair side and the contralateral, control side showed no difference between tibialis anterior compound muscle action potentials of the autograft (41.9%) in six repairs and the poly-DL-lactide-ε-caprolactone conduit (36.2%) in eight repairs, which is also in agreement with our results. A distance-related effect on reinnervation was also observed, with a lower rate and amount of recovery of the plantar muscle compound muscle action potentials. In our study, better results for compound muscle action potential were observed in the tibialis anterior compared with the flexor digiti quinti brevis muscle, which may be attributed to the shorter distance of the tibialis anterior to the repair. Furthermore, the size of the severely atrophied flexor digiti quinti brevis may have surpassed the lower limit for reliable compound muscle action potential measurements in our study. It is possible that the longer distance of the flexor digiti quinti brevis to the repair resulted in more irreversible motor unit changes, or the muscle was still in the process of reinnervation and regeneration at the time of measurement.

Axon counts distal to the repair with the autograft and the poly-DL-lactide-ε-caprolactone conduit were found to be increased compared with the control side (118% and 112%, respectively), which is associated with the improved muscle force recovery seen in those two groups. A nonsignificant decrease in peroneal nerve area, average fiber area, and myelin thickness was observed in all repair types (Table I). This suggests that the magnitude of motor recovery is a function of the number of regenerating axons rather than nerve area, nerve fiber size, or amount of myelination. Similarly, Archibald et al. observed an increase in the number of myelinated axons and a decrease in total fascicular area and fiber diameter compared with controls in monkeys following successful nerve gap repair with an autograft and a type-I-collagen conduit after two to four years[32]. An increase in fiber density and a reduction in fiber diameter were also observed by Den Dunnen et al. in rats two years after a repair of an 8-mm sciatic defect with a poly-L-lactide-ε-caprolactone conduit[39]. Clavijo-Alvarez et al. observed reduced myelination distal to a nerve repair in aged (eleven-month-old) rats with use of an autograft and conduits of polyglycolic acid, polycaprolactone, and collagen-embedded polycaprolactone[40]. Similar to our results, no significant difference was found among groups.

The poor motor outcomes of the polyglycolic acid conduit (group IV) are contrary to the positive clinical observations of motor recovery reported in humans[18,20,21,24]. This disparity may be due to a number of reasons: high degradation rate, nerve-lumen size mismatch, animal model, and/or healing rate. First, an excessive rate of degradation of the polyglycolic acid material by hydrolysis may have existed in our model. Clavijo-Alvarez et al. observed a higher degradation rate in the polyglycolic acid conduit compared with fabricated polycaprolactone and collagen-embedded polycaprolactone conduits[40]. In our study, structural failure was observed in all polyglycolic acid conduits at twelve weeks. The conduits had lost their tubular rigidity and had completely collapsed (Fig. 3), which may have resulted in nerve compression, inhibiting regeneration across the repair. Conversely, both the poly-DL-lactide-ε-caprolactone and type-I-collagen conduits had retained their tubular structure at twelve weeks (Figs. 1 and 2). Heparinized saline solution was not injected into any of the conduits during repair, which may have contributed to degradation, scar formation, and structural failure. Twice-daily injections of heparin over three days in addition to a single localized dose have been observed to depress wound inflammation and edema in rats after nerve conduit repair[41]. Heparin can be used to prevent the formation of blood clots, which may act as a mechanical impediment to the axonal growth cone[16]. The formation of a blood clot may have been the mechanism for the single case of reinnervation failure seen in the poly-DL-lactide-ε-caprolactone group. However, no blood clot was found in the lumen of any of the polyglycolic acid conduits at the time that the rats were killed. The use of the polyglycolic acid conduit has previously been investigated in rats with varying degrees of success, but the use of heparin was not mentioned in the two previous studies[37,40]. Second, a mismatch in nerve and conduit diameter may have played a role in the poor regeneration rate of the polyglycolic acid conduits. The smallest available diameter was 2.3 mm. The 1.5-mm inner diameter of the poly-DL-lactide-ε-caprolactone and type-I-collagen conduits more closely matched the size of the rat sciatic nerve. In a review of the case reports, it was found that the polyglycolic acid conduit had been used in facial nerve as well as spinal accessory nerve reconstruction—both of which would represent a substantial size mismatch[18,21]. It is understood that the nerve conduit must be of greater diameter than the nerve to allow for swelling of the nerve ends[5,31]. However, further study may be needed to determine if an upper limitation exists for the greater diameter of the conduit compared with the nerve, which may explain our observations. Third, as discussed by Waitayawinyu et al., this poor performance may be unique to the rat model compared with primates and humans[37]. Lastly, the polyglycolic acid conduit may have a slower initial recovery rate than the other methods of repair, which would be made apparent at our short recovery period of twelve weeks. Waitayawinyu et al. reported a 42% rate of recovery of tibialis anterior tetanic muscle force at fifteen weeks compared with 3% at twelve weeks in our study[37].

The methods of evaluation of motor outcomes following segmental nerve repair need to be carefully considered. For example, walking track analysis has been used to evaluate functional motor nerve recovery in the rat[42]. However, the development of chronic joint contractures was found to invalidate the walking track method for long-term assessment of nerve repair[43]. Urbanchek et al. highlighted the lack of correlation between walking track and maximum muscle force of the extensor digitorum longus following sciatic nerve injury

and repair in the rat, suggesting different factors were being evaluated[44]. For this reason, we did not perform a walking track assessment in this study. Furthermore, a poor correlation between electromyographic and clinical assessments has been observed following nerve repair in children[26]. In our study, functional recovery was directly assessed by measurement of the maximum isometric tetanic force production of the tibialis anterior and estimated by measurements of compound muscle action potential, muscle weight, and histology. Interestingly, the relative comparisons between repair types by all methods of analysis (compound muscle action potentials, muscle force, muscle weight, and axon counts) were in general agreement with each other. However, care must be taken when interpreting the results of these different motor recovery outcomes as they evaluate different aspects of the motor unit.

This study was limited by the single end point of twelve weeks. More time is needed to assess long-term motor outcomes when the three different nerve conduit materials are compared. Multiple time points would be useful in observing long-term trends of motor recovery, but this is difficult to accomplish without a large number of rats because of the inability to make serial measurements of direct muscle force, muscle weight, and nerve histomorphometry in the animals.

Even with these limitations, this study has clearly demonstrated that the autograft and the poly-DL-lactide-ε-caprolactone conduit were similar at twelve weeks with respect to the recovery of compound muscle action potentials, muscle force, muscle weight, and axon count. The polyglycolic acid conduit had the poorest recovery of compound muscle action potentials and maximum isometric tetanic muscle force as well as mean recovery of all assessments. The motor outcomes of the type-I-collagen conduit fell between that of the poly-DL-lactide-ε-caprolactone and polyglycolic acid conduits. These results demonstrated the functional regenerative potential and pitfalls of bioabsorbable synthetic nerve conduits in segmental motor nerve repair, and they demonstrated that there are clear differences between available conduits with respect to motor nerve recovery in the rat model. ■

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