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# Peripheral nerve regeneration through a synthetic hydrogel nerve tube

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**Abstract**. *Purpose:* As alternatives to nerve grafts for peripheral nerve repair, we have synthesized 12 mm long poly(2-hydroxyethyl methacrylate-co-methyl methacrylate) (PHEMA-MMA) porous tubes and studied their regenerative capacity for the repair of surgically-created 10 mm rat sciatic nerve gaps. We compared the *in vivo* regenerative efficacy of these artificial tubes with the gold standard, the nerve autograft.
*Methods:* Tubes were assessed *in vivo* for their ability to support nerve regeneration at 4, 8, and 16 weeks post-implantation by histology, electrophysiology, histomorphometry, and reinnervated lateral gastrocnemius (LG) dry muscle mass.
*Results:* Axonal regeneration within the tubes was observed by 8 weeks, with outcome parameters comparable to autografts. This finding was further supported by the electrophysiological and histomorphometric results. The 16 week tube group had a bimodal response, with 60% of the tubes having a similar response to autografts and the other 40% having significantly lower ($p < 0.05$) outcome measures in several parameters.
*Conclusions:* Axonal regeneration in artificial tubes was similar to that in autografts at 8 and 16 weeks, however, a bimodal distribution of regeneration was observed in 16 week tubes.

Keywords: Functional outcome measures, morphometry, poly(2-hydroxyethyl methacrylate-co-methyl methacrylate), rat, sciatic nerve

## 1. Introduction

Peripheral nerve injury (PNI) affects up to 2.8% of trauma patients, many of whom incur life-long disability [31]. Approximately 360,000 Americans suffer from upper extremity paralytic syndromes yearly, resulting in 8,648,000 restricted activity days and 4,916,000 bed/disability days [16]. Furthermore, 44,000 upper extremity inpatient procedures involved the nervous system in the United States from 1989 – 1991 [16].

The gold standard for repairing peripheral nerve injury gaps longer than 5 mm is the nerve autograft [29]. However, there are many disadvantages and complications with using autografts for nerve reconstruction. A secondary injury is made in order to repair the primary one. Scar and occasional neuroma pain can result form donor site morbidity [32]. Another obstacle is insufficient donor tissue availability, as the autograft material may be of insufficient length and diameter to optimize the repair [21]. Modern microsurgical techniques used to approximate the two stumps of a transected nerve

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have reached an optimum [5]. Results achieved using a nerve autograft vary from extremely poor [19] to very good [28]. The basal lamina-lined endoneurial tubes of a grafted nerve are oriented in a linear fashion and can impose non-topographic directionality to a regenerating nerve axon, leading to non-specific and incomplete reinnervation of the distal nerve stump giving rise to poor functional recovery [8].

A bioengineered graft bridging the proximal and distal nerve stumps would prevent the need for a second surgery (and the implications derived as a result of the procedure). Many of the graft properties (such as length, diameter, and rigidity) can be manipulated to meet clinical requirements. Furthermore, the regenerative capacity of synthetic nerve tubes can be enhanced with the incorporation of growth factors into the lumen [26].

Various nerve conduits have the capacity to permit peripheral nerve regeneration. However, collapse, scar infiltration, and early resorption are problems that can occur over long gaps that limit the conduit's potential for promoting nerve regeneration [10]. Moreover, cytotoxic degradation products, released from biodegradable materials during the resorption process, cause a secondary inflammatory response in terms of a substantial macrophage invasion, fibrosis, and disorganized axonal growth.

We describe herein the regenerative capacity of 12 mm long, porous tubular grafts, composed of poly(2-hydroxyethyl methacrylate-co-methyl methacrylate) (PHEMA-MMA), for the repair of surgically created 10 mm rat sciatic nerve gaps at 4, 8, and 16 weeks post-implantation. We use a novel, reproducible process to create concentric hydrogel nerve tubes that allows precise control of their dimensions, morphology, and mechanical properties [6]. Our goal is to compare the axonal regeneration and functional outcome achieved with PHEMA-MMA hydrogel tubes relative to autografts. Hydrogels have been used for numerous biomedical applications: soft contact lenses, artificial tendon materials, wound-healing bioadhesives, artificial kidney membranes, articular cartilage, artificial skin, and drug delivery gels (reviewed in [33]).

## 2. Materials and methods

### *2.1. Tube preparation*

All chemicals were purchased from Aldrich Chemicals (Milwaukee, WI) and were used as received unless otherwise stated. Hydrogel nerve tubes were manufactured using a technique developed by Dalton and Shoichet as previously described [7]. The PHEMA-MMA tubes utilized in this study were composed of 33% monomer of which 86% was HEMA and 14% was MMA by mass. The hydrogel nerve tubes had an inner diameter of 1.3 mm, an outer diameter of 1.8 mm, and a length of 12 mm. Pilot studies in our lab showed that an inner tube diameter of 1.3 mm is large enough to allow for nerve stump swelling without compressing the nerve. Tube cross-sections of 100 $\mu$m thickness were taken from each end of the 12 mm long tubes and viewed under the stereomicroscope (Leica MZ-6, Leica Microsystems, Wetzlar, Germany) in order to verify tube concentricity. The tubes were Soxhlet extracted in water overnight, sterilized by autoclaving in phosphate buffered saline (PBS), and then filled with sterile-filtered 1.28 mg/ml type I collagen, as previously described in [26].

### *2.2. In vivo implantation and study design*

Inbred adult male Lewis rats (250–275 g) were obtained from Harlan Sprague Dawley (Indianapolis, IN). Standard microsurgical techniques were used with the assistance of an operating microscope (Leica M651, Leica Microsystems, Wetzlar, Germany) [27]. All experiments and animal interventions strictly adhered to Canadian Council on Animal Care guidelines. The anaesthesia consisted of an intramuscular injection of 10 mg/kg xylazine (20 mg/ml; Bayer Inc., Etobicoke, ON) and 100 mg/kg ketamine hydrochloride (0.1 ml/100 g Rogarestic; Rogra-STB, Montreal, QC) into the lumbar paraspinal musculature. Surgical sites were shaved after the intramuscular anaesthetic injection and prepared with Betadine and 70% surgical alcohol. Following gluteal and posterior thigh incisions, the sciatic nerve was exposed deep to the biceps femoris muscle and an 8 mm segment of the nerve was excised which, after retraction of the nerve ends, resulted in a 10 mm nerve gap. A 12 mm long PHEMA-MMA tube was sutured into the resulting gap using two 10-0 nylon sutures (Dermalon, Davis and Geck, American Cyanamid Company, Danbury, CT) on each side approximately 180° apart. Eight sciatic nerve graft segments (10 mm long) were harvested from a total of eight sides (right and left) from four isogenic Lewis donor rats. The donor animals were then euthanized. The harvested nerve graft segments were repaired into 10 mm long gaps surgically created in recipient rats and repaired with 10 – 0 nylon epineurial sutures similar to

the tube repair. Muscle and skin incisions were approximated with interrupted 3 – 0 Polysorb (Autosuture, Norwalk, CT) and continuous 3 – 0 silk (Autosuture, Norwalk, CT) sutures, respectively.

Each rat underwent bilateral surgery. In many cases, a tube was implanted on one side and a nerve graft on the other. In some groups, where there were more tubes than autografts, tubes were implanted on both sides. Three rats (3 autografts, 3 tubes) were devoted to the 4 week neurofilament 200 immunoreactivity (NF200 IR) outcome group, 8 rats (8 autografts, 8 tubes) to the 8 week NF200 IR outcome group, 5 rats (4 autografts, 6 tubes) to the 8 week histomorphometry/electrophysiology outcome groups, and 11 rats (4 autografts, 18 tubes) to the 16 week electrophysiology/muscle mass outcome groups. Seven rats (4 autografts, 10 tubes) of the latter 11 were also used for the 16 week histomorphometric analysis. The tube/autograft allocation plan is summarized in Table 1.

### *2.3. Electrophysiology*

At 8 and 16 week end-points, electrophysiological studies were performed prior to tissue harvesting. Bipolar hooked platinum electroencephalographic stimulating electrodes were placed under the sciatic nerve 10 mm proximal to the injury site [23]. Recording electrodes recorded supramaximal evoked responses from the distal (to repair) sciatic nerve and reinnervated gastrocnemius muscle, and were placed 30 mm and 40 mm distal to the stimulating electrodes, respectively. Measurements were verified with the use of a calliper and a ruler. A ground electrode was placed in a superficial muscle layer near the skin. Four mean parameters were measured: 1) nerve action potential (NAP) conduction velocity, 2) NAP amplitude, 3) area under the NAP curve, and 4) muscle action potential (MAP) conduction velocity. These measurements were obtained by a computer-assisted electromyographic machine (Cadwell 6200A, Cadwell Laboratories Inc., Kennewick, WA). Conduction velocities were calculated from derived latencies and measured distances.

### *2.4. Neurofilament 200 immunoreactivity*

The graft (autograft or tube) and its contained regenerating nerve cable were analysed in the proximal and distal segments using immunohistochemical methods at 4 and 8 weeks post-implantation for neurofilament 200 (NF200) proteins. NF200 is a commonly-used marker for axonal outgrowth preferentially staining axonal neurofilaments [34–36].

The anaesthetized rats were perfused through the heart with saline followed by 4% ice cold paraformaldehyde. These rats were used solely for NF200 analysis. Harvested proximal and distal tube samples (along with the nerve tissue immediately proximal and distal, respectively) were cryopreserved in 30% sucrose solution for 24 hours and frozen in a Cryomatrix compound. Longitudinal sections were cut at 15 $\mu$m on a cryostat. Approximately 5 longitudinal sections from each sample were taken for a qualitative analysis. Slides were dewaxed in three changes of xylol, dehydrated in two changes of 100% ethanol, and rinsed with a solution of PBS, 0.03% hydrogen peroxide and 1% sodium azide. Slides were treated with 0.2% pepsin solution in Tris Buffered Saline (TBS) at 37°C for 15 minutes, rinsed in tap water, PBS, and blocked with 7% normal horse serum for 15 minutes, and then incubated with monoclonal mouse anti-human NF200 (Clone RT97, Novacastra Laboratories, Burlingame, CA, USA) at room temperature for 1.5 hours at a dilution of 1/500. Sections were washed three times in TBS before and after the 45 minute secondary antibody (horse anti-mouse biotinylated IgG, BA 2001) application. Commercial Vectastain ABC Kit (PK 4000, Vector Laboratories, Burlington, ON, Canada) was applied and incubated for 40 minutes before rinsing with TBS and the addition of the chromagen solution (SK 4800, Vector Laboratories). Slides were washed for 5 minutes in running tap water, lightly counterstained in Mayers hematoxylin (as described in [17]), dried overnight, and coverslipped using Permount (Fisher Scientific).

### *2.5. Histomorphometry*

Approximately 4 mm long fresh tissue samples from 8 and 16 week tube and nerve autograft mid-portions and 5 mm distal to the distal suture line were harvested, underwent glutaraldehyde fixation, plastic embedding, and were sectioned on an ultramicrotome (Sorvall MT6000, Ivan Sorvall, Inc., Norwalk, CT). These 1 $\mu$m thick cross-sections were stained with Toluidine Blue and viewed under the light microscope. One cross-section from the centre of each sample was taken into consideration for statistical analysis.

Seven representative non-overlapping high power fields (HPFs) of view (3093 $\mu m^2$, at a magnification of X1000) were selected from each sample and were examined for myelinated axons greater than 1 $\mu$m in diameter. These fields were taken from all aspects

Table 1
Final nerve and tube graft allocation

| | 4 weeks | | 8 weeks | | 16 weeks | |
|---|---|---|---|---|---|---|
| | Autograft | Tube | Autograft | Tube | Autograft | Tube |
| Neurofilament IR[1] | 3 | 3 | 8 | 8 | | |
| Histomorphometry | | | 4 | 6 | 4 | 10 |
| Electrophysiology | | | 4 | 6 | 4 | 17* |
| Muscle Mass | | | | | 4 | 17* |

[1]IR = Immunoreactivity.
*Initially, 18 tubes were implanted for 16 week electrophysiology and muscle mass analyses, but suture pull-out occurred in the distal end of one of them which prevented end-point evaluation.

of the regenerating cable and excluded the tube wall, epineurium, and large blood vessels. The distribution of axons in both tubes and nerve grafts was relatively homogeneous. The seven HPFs per sample comprised 13–27% of the total nerve regenerating cable area. With the aid of the Image Pro-Plus software program, a colour-intensity-based method (described in [26]) identified all the healthy myelinated nerve fibres within each HPF.

The histomorphometric analysis was completed on 8 and 16 week mid-graft and distal nerve portions for mean: 1) fibre diameter (diameter of the entire myelinated axon); 2) G ratio (axon diameter: fibre diameter – to assess nerve fibre maturity); 3) total myelinated axonal count (an estimation derived from count, sampled area, and the measured fascicular area); 4) axon diameter; 5) myelin thickness; 6) axon: myelin area; 7) fibre count in 7 HPFs per sample; 8) nerve cross-sectional area; 9) axonal density; and 10) neural tissue percentage [26,30].

### 2.6. Lateral gastrocnemius dry muscle mass

The lateral gastrocnemius (LG) muscle was excised after 16 weeks post-implantation in tube and autograft group animals to assess the degree of reinnervation [11, 12]. The tissue was blotted dry and weighed on an electronic scale (Mettler AJ100, Mettler Instruments, Greifensee-Zurich, Switzerland).

### 2.7. Statistical analysis

Each of the dependent variables from the morphometric, histomorphometric, electrophysiologic, and muscle mass variables were analyzed separately. Mean values from each time point and group (autograft or tube repair) for each dependent variable were compared using independent sample t-tests to compare 2 groups using a 95% confidence interval. ANOVAs were conducted to compare the three 16 week groups: autografts, tubes, and the tube sub-group. This sub-group was comprised of six 16 week tubes that contained regenerating cables. If the ANOVA demonstrated significant ($p < 0.05$) overall effects, then specific mean comparisons were performed for that variable with post hoc independent sample t-tests, with a 95% confidence level of significance, using the Scheffe method [37]. The statistical software program used was STATISTICA for Windows (StatSoft, Inc. (1998), Tulsa, OK).

## 3. Results

### 3.1. General histological evaluation

PHEMA-MMA tubes, which bridge a 10 mm gap in the sciatic nerve, support regeneration of a nerve cable and are biocompatible at 8 weeks [3,25]. As evidenced in Fig. 1, after 8 weeks of implantation, the regenerating cable tapered relatively little from the inner aspect of the tube and seemed to penetrate the inner spongy polymeric tube wall (Fig. 1(A)). The sciatic nerve 5 mm distal to the tube was fasciculated and well-vascularized (Fig. 1(B)). After 16 weeks of implantation, a tissue cable was observed in the mid-portion of the tube (Fig. 2(A)). Within the regenerating cable, the size, shape, number and myelin area of the myelinated axons indicated substantial regeneration (Fig. 2(B)). The cable was also abundant with unmyelinated nerve fibres and blood vessels (Fig. 2(B)). At week 16, the microfasciculation observed in the nerve 5 mm distal to the tubes was not as pronounced as that observed in the distal nerve section beyond the nerve autograft repair. In the autograft controls, there was robust regeneration in all the mid-graft samples at both 8 and 16 weeks, and a greater degree of microfasciculation and vascularization at the later time point. Six of the ten 16 week PHEMA-MMA synthetic tubes supported a similar regeneration as autograft controls. Four of the 10 samples lacked a regenerating cable at 16 weeks likely because 3 of these 4 tubes collapsed by at least 90% of their original (pre-implantation) tube outer diameter.

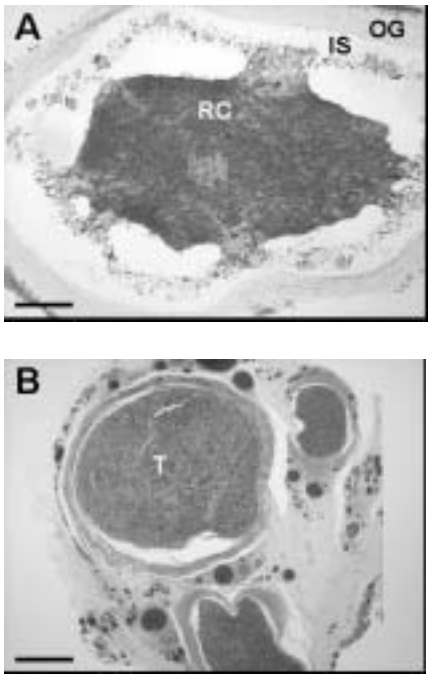


Fig. 1. Representative photomicrographs of 1 $\mu$m Toluidine-blue stained cross-sections of 8 week A) mid-tube and B) distal nerve samples. A) Low-power photomicrograph of a typical 8 week tube and its contained regenerating cable (RC). Note the tube was comprised of two phases: an inner spongy (IS) and an outer gel (OG) layer. B) A cross-section of the sciatic nerve 5 mm distal to the distal suture line of the 8 week tube graft. The fascicle used for histomorphometric analysis was the tibial (T) fascicle. Scale bars are 200 $\mu$m.

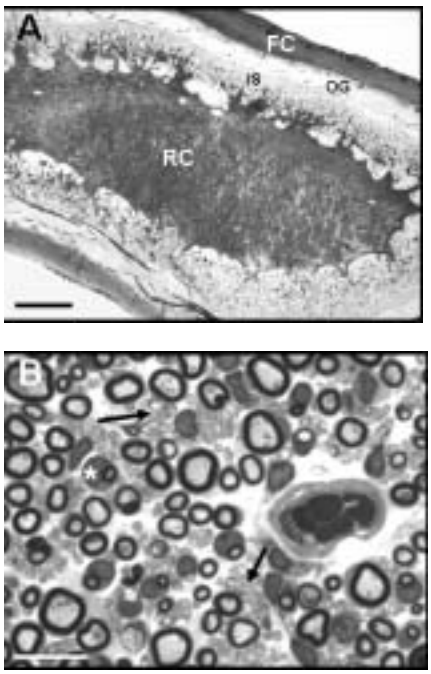


Fig. 2. Representative photomicrographs of 1 $\mu$m Toluidine-blue stained cross-sections of a 16 week tube (mid-graft level) at A) low-power and B) high-power magnification. A) Low power photomicrograph of a 16 week tube with its IS and OG layers, contained RC and an external vascularized fibrous capsule (FC). Scale bar is 200 $\mu$m. B) The regenerating cable is abundant with unmyelinated fibres (arrows) and adequately myelinated fibres. Schwann cells (*) and a blood vessel can also be found in this photomicrograph. Note the axons are not compartmentalized. Scale bar is 10 $\mu$m.

### *3.2. Neurofilament 200 analysis*

Neurofilament analysis of axons was used to gauge regeneration in PHEMA-MMA tubes versus autograft controls. While NF200+ axons were present at the distal-graft level in all autografts after 4 weeks of implantation, none were observed in the distal graft level of PHEMA-MMA tubes until 8 weeks after implantation. At 8 weeks, 4 of the 8 (50%) tubes did show NF200+ axons linearly oriented at the proximal tube level (Fig. 3(A)), with good axonal regeneration in their corresponding distal tube segments (Fig. 3(B)). The other tube-repaired rats had poor axonal regeneration at the proximal graft level (compare non-linearly oriented NF200+ axons observed as in Fig. 3(C)), as well as no regeneration into their corresponding distal tube segments (Fig. 3(D)).

### *3.3. Histomorphometry*

At 8 weeks post-implantation there were no significant differences ($p > 0.05$) between the autograft- and the tube-repaired animals in the mid- or distal-levels for eight of the ten histomorphometric parameters measured – fibre diameter, G ratio, axon diameter, myelin thickness, axon-to-myelin ratio, HPF fiber counts, nerve area, and axonal density (Table 2). Only the total axon counts and the neural tissue percentage at the mid-graft level were statistically lower in tubes than in autograft repairs. These significant differences, however, were not evident in the distal nerve level.

At 16 weeks post-implantation, there were no significant differences ($p > 0.05$) between the autograft- and the tube-repaired animals in the mid- or distal-levels for seven of the ten histomorphometric parameters measured – fibre diameter, G ratio, axon diameter, myelin thickness, axon-to-myelin ratio, nerve area, and neural tissue percentage (Table 3). However, at 16 weeks, the entire tube-repaired group had significantly fewer ($p < 0.05$) axon counts, HPF fibre counts, and a lower axonal density in the mid-graft portions (Table 3). It is interesting to note that when autografts were compared to tubes with regenerating cables (RC) – that is, the 6

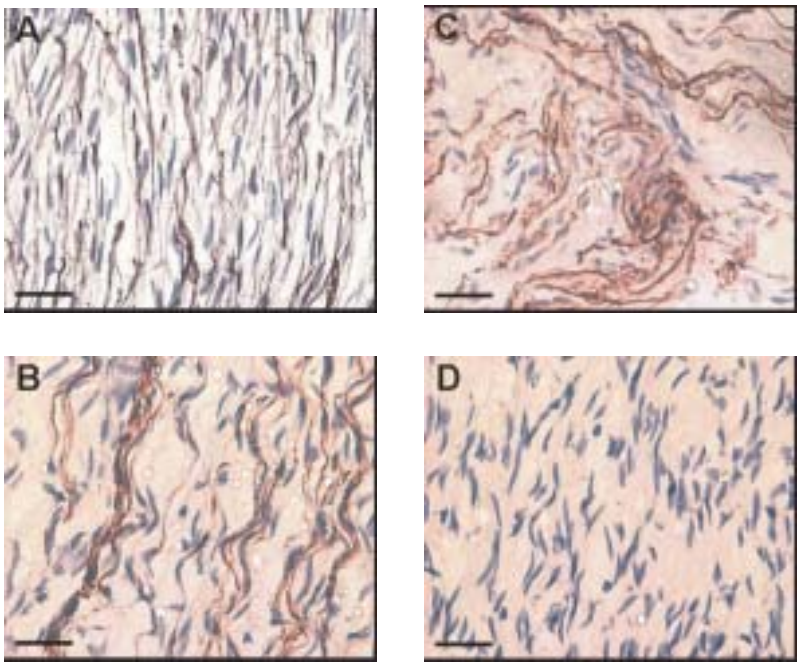


Fig. 3. Photomicrographs of longitudinal NF200-stained sections at 8 weeks post-implantation. A) Properly linearly aligned NF200+ axons were found in half of the 8 week proximal tube samples, as shown here, and they were similarly found in their B) corresponding distal tube regions. C) Some of the proximal tube samples at 8 weeks displayed NF200+ axons that were haphazardly oriented, as demonstrated here, with their D) corresponding distal tube portions devoid of axons. In these photomicrographs, nuclei are blue and the neurofilaments of axons are reddish-brown. Scale bars are 20 $\mu$m.

out of 10 tubes – at 16 weeks, there was no statistical difference in any of the histomorphometric parameters. Moreover, as in the 8 week histomorphometric analysis, there were no significant differences in any of the ten parameters at the distal nerve level in the 16 week group although the autograft group had higher means than the tube group.

### *3.4. Electrophysiology*

At 8 weeks post-implantation, there were no significant differences between the tube and the autograft groups for the mean nerve action potential (NAP) conduction velocity, NAP amplitude, area under NAP curve, and muscle action potential (MAP) conduction velocity (Figs 4(A–D)). At 16 weeks post-implantation, there were no significant differences between the tube group (with regenerating cables) and the autografts in terms of the mean NAP conduction velocity and area under NAP curve (Fig. 4(E,F)). However, the mean NAP amplitude and MAP conduction velocity were significantly lower ($p < 0.05$) in the tube sub-group compared to the autograft group (Fig. 4(G,H)). When the entire tube group was compared (including the subset of tubes without a regenerating cable), the mean NAP amplitude, area, and the MAP conduction velocity were significantly lower ($p < 0.05$) in the tube group relative to autografts at 16 weeks.

### *3.5. Lateral gastrocnemius dry muscle mass*

The 16 week mean LG mass of the tube group was significantly ($p < 0.05$) lower than that of the autograft group, even when the autografts were compared with the subset of tubes with regenerating cables (Fig. 5).

## 4. Discussion

In nerve conduit studies, within the first week, a matrix coalesces (consisting largely of fibrin polymers) that is relatively acellular [38]. This fibrin matrix provides a scaffold for the migration and seeding of cells from both nerve stumps during the second week. The formation of this fibrin matrix is critical for regeneration. If a matrix fails to form, as can happen when a tube is used to repair a long gap, no regeneration will occur. The thickness and quality of the fibrin matrix can be influenced by the dimensions of the tube. The

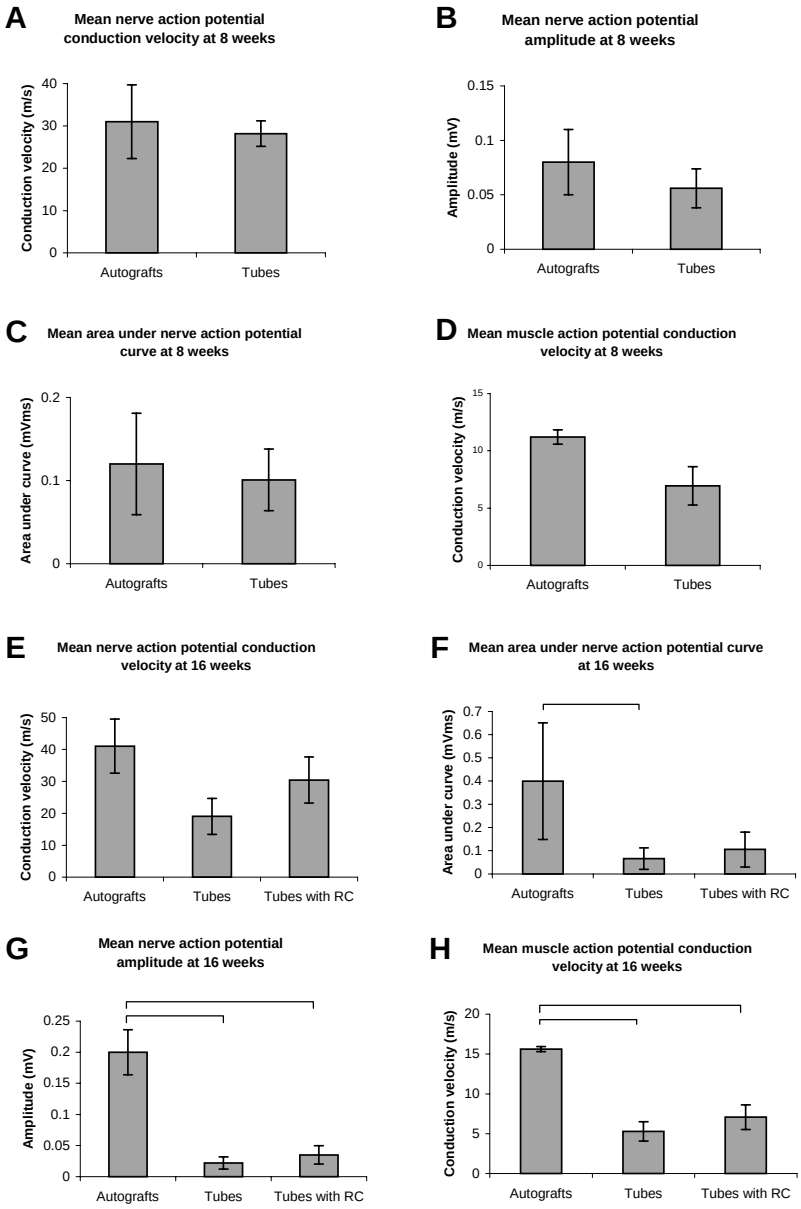


Fig. 4. There were no statistical differences between the 8 week tube- ($n = 6$) and autograft-repaired ($n = 4$) groups in A) nerve action potential (NAP) conduction velocity, B) NAP amplitude, C) area under the NAP curve, and D) muscle action potential (MAP) conduction velocity. One 8 week tube-repaired rat side out of 6 (17%) exhibited negative (that is, zero) responses in all four electrophysiologic parameters and was included in the results. Though the mean NAP conduction velocity was similar between the autograft ($n = 4$) and entire tube ($n = 17$) groups in E) at 16 weeks, the other three 16 week parameters (F–H) showed statistical differences ($p < 0.05$) between these two groups. When comparing the tube group with regenerating cables (RC; $n = 6$) to the autograft group, only 2 of the 4 electrophysiological parameters, G) NAP amplitude and H) MAP conduction velocity, showed significant differences ($p < 0.05$). Connecting lines above the bars indicate significant differences ($p < 0.05$). Three tube-repaired rats (part of the entire tube group, but not the "Tubes with RC" group) out of 10 (30%) showed negative (that is, zero) responses in these four electrophysiologic parameters which were included in the results. Error bars denote SEM.

regenerating cable often tapers from both the proximal and distal nerve stumps towards the mid-tube area and this tapering constrains axonal regeneration [18]. While tapering is usually problematic in gap lengths longer than those studied herein, it is important to note that we observed little to no tapering by general histologic inspection. The regenerating cable appeared to comprise more of the interior of the lumen area as

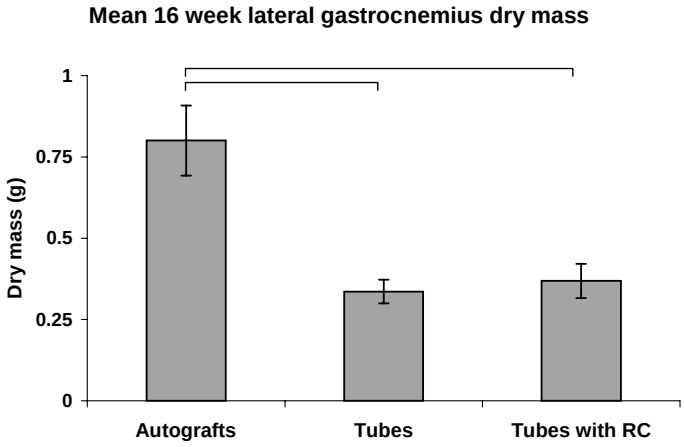


Fig. 5. Lateral gastrocnemius (LG) dry muscle mass assessment at 16 weeks. The entire tube-repaired group ($n = 17$) as well as the tube sub-group with regenerating cables (RC, $n = 6$) had significantly lower lateral gastrocnemius (LG) dry mass means compared to the autograft group ($n = 4$) (ANOVAs and post hoc independent sample t-tests using the Scheffe method ($p < 0.05$)). Error bars denote SEM.

compared to synthetic conduits from other studies [18]. Furthermore, the nerve cross-sectional areas in the mid-portion of tubes and autografts at both 8 (Table 2) and 16 (Table 3) weeks were not statistically different from each other. The observation of a nerve of considerable size within the lumen of the tube may be due to the cell-invasive morphology of the tube wall [2,3]. It is unknown whether the lack of regenerating cables in some 16 week tubes was caused by a lack of fibrin matrix formation in the initial stages of regeneration or the degeneration of a formed regenerating cable. To answer this point, shorter term studies with the PHEMA-MMA tubes must be conducted.

The 4 week NF200 results demonstrate that the regeneration rate may be delayed in the tube group compared to the autografts which has also been reported elsewhere [18,38]. However, a more comprehensive study must be completed at earlier timepoints to investigate the temporal progression of axons from the proximal to the distal stumps.

At 8 weeks, the tube-repaired animals performed similarly to the autograft-repaired animals in all four electrophysiological parameters tested and in all of the ten histomorphometric parameters at the distal nerve level. The NF200 staining presented a general qualitative impression of the longitudinal organization of axons in the samples. The Toluidine Blue cross-sections provided a much better estimate of regeneration through the tubes because longitudinal sections may misrepresent the axons present, but not in that section. While some longitudinal tube sections lacked NF200 IR, it does not necessarily mean the sample was devoid of axons; it may be merely a reflection of where the longitudinal section was taken. Cross-sectional samples, such as the ones used to draw the histomorphometry results, were more representative.

At 16 weeks post-implantation, a bimodal distribution of regeneration was observed in tube-repaired animals. In those tubes that had a regenerating cable (that is, 60% at 16 weeks), the results were similar to those of the autograft. There was a strong correlation between tubes lacking a regenerating cable (40% of 16 week tubes) and tube collapse, suggesting that this problem could be overcome by engineering a tubular structure that remains patent during the course of regeneration. Alternatively, these PHEMA-MMA tubes may be used to bridge very short defects, for instance, as alternatives to conventional repair.

The 8 week G ratios of the fibres from both tube and autograft repairs deviated slightly from the normal range of 0.50 to 0.55 [9,13]; however, by 16 weeks, the G ratios converged nearer to this range in those grafts that had regenerating cables, representing good progression to maturation.

Histomorphometrically, there was no statistical difference between autografts and tube grafts. However, the number of nerve fibres (and the parameters related to it) was different in the tube- and autograft-repaired animals. The greater numbers of nerve fibers in the autografts may account for differences observed in the electrophysiologic and muscle mass measurements reflecting better end-organ reinnervation in the autograft group. It should be noted that the lack of statistical differences in some cases may be attributable to a low sample size.

Table 2
Eight week histomorphometry data means (and standard errors of the means – SEMs)

| | Mid-graft | | Distal Nerve | |
|---|---|---|---|---|
| Parameters | Autograft | Tube | Autograft | Tube |
| Fibre Diameter ($\mu$m) | 3.51 (0.01) | 3.22 (0.05) | 3.22 (0.11) | 2.42 (0.49) |
| G Ratio | 0.63 (0.03) | 0.63 (0.03) | 0.52 (0.01) | 0.43 (0.09) |
| Total axon counts | 8572 (1191)* | 4824 (1007)* | 2343 (142) | 1987 (548) |
| Axon Diameter ($\mu$m) | 2.22 (0.09) | 2.03 (0.09) | 1.66 (0.06) | 1.24 (0.26) |
| Myelin Thickness ($\mu$m) | 0.64 (0.05) | 0.59 (0.05) | 0.78 (0.03) | 0.59 (0.12) |
| Axon: Myelin | 0.736 (0.005) | 0.738 (0.032) | 0.567 (0.037) | 0.481 (0.108) |
| Fibre Count 7 HPFs[1] | 425.50 (30.88) | 373.00 (80.53) | 237.25 (38.59) | 169.50 (41.72) |
| Nerve Area (mm$^2$) | 0.433 (0.041) | 0.376 (0.098) | 0.228 (0.033) | 0.218 (0.059) |
| Density (/ mm$^2$) | 19653 (1426) | 17228 (3719) | 10958 (1782) | 7829 (1927) |
| Neural Tissue % | 0.220 (0.008)* | 0.185 (0.006)* | 0.204 (0.015) | 0.138 (0.030) |

[1]HPFs = High Power Fields.
*significantly different ($p < 0.05$); independent sample t-test.

Table 3
Sixteen week histomorphometry data means (and SEM)

| | Mid-graft | | | Distal Nerve | | |
|---|---|---|---|---|---|---|
| Parameters | Autograft | Tube | Tubes with RC[1] | Autograft | Tube | Tubes with RC[1] |
| Fibre Diameter ($\mu$m) | 3.70 (0.14) | 2.21 (0.61) | 3.68 (0.13) | 3.46 (0.22) | 1.96 (0.54) | 3.27 (0.16) |
| G Ratio | 0.55 (0.02) | 0.34 (0.01) | 0.57 (0.03) | 0.52 (0.02) | 0.29 (0.08) | 0.49 (0.01) |
| Total Axon Counts | 9193 (2032)* | 2577 (1097)* | 4295 (1460) | 2022 (544) | 916 (425) | 1526 (597) |
| Axon Diameter ($\mu$m) | 2.03 (0.06) | 1.26 (0.35) | 2.10 (0.11) | 1.77 (0.18) | 0.97 (0.27) | 1.61 (0.10) |
| Myelin Thickness ($\mu$m) | 0.83 (0.06) | 0.47 (0.13) | 0.79 (0.07) | 0.85 (0.03) | 0.50 (0.14) | 0.83 (0.04) |
| Axon: Myelin | 0.593 (0.035) | 0.378 (0.107) | 0.631 (0.049) | 0.511 (0.053) | 0.299 (0.083) | 0.499 (0.030) |
| Fibre Count 7 HPFs[2] | 458.00 (13.65)* | 174.00 (58.08)* | 290.00 (58.22) | 258.33 (15.65) | 113.00 (44.23) | 188.33 (55.07) |
| Nerve Area (mm$^2$) | 0.430 (0.082) | 0.230 (0.089) | 0.384 (0.109) | 0.176 (0.058) | 0.097 (0.033) | 0.162 (0.034) |
| Density ( / mm$^2$) | 21154 (630)* | 8037 (2682)* | 13394 (2689) | 11932 (723) | 5219 (2043) | 8699 (2543) |
| Neural Tissue % | 0.273 (0.024) | 0.158 (0.059) | 0.263 (0.033) | 0.245 (0.027) | 0.133 (0.050) | 0.222 (0.027) |

[1]Tubes with RC = Tubes with Regenerating Cables.
[2]HPFs = High Power Fields.
*significantly different ($p < 0.05$); independent sample t-tests.

The normal rat sciatic nerve contains 7115 $\pm$ 413 myelinated nerve fibres [20]. At 8 and 16 weeks, the autograft group at mid-graft level had higher than normal counts, which is in agreement with previous peripheral nerve grafting studies [1,15,20,22], likely due to axonal sprouting [14]. The tube group, on the other hand, had significantly lower counts at mid-tube level than the autograft group at 8 and 16 weeks (Tables 2 and 3). While there are numerous factors that may account for the lower axonal counts in the tube group compared to the autograft group, the most likely is the apparent compression observed in some of the 16 week tubes [10]. When tubes with no regenerating cables were excluded from the analyses, the results were much more optimistic. Other factors that have been described previously include: the pruning of misdirected axons [20], progressive decrease of axonal numbers across the tube distally [15], loss of axons at the suture lines [22], and inflammation [5]. Collectively, these factors may have reduced the number of peripheral connections made at the targets and thereby worsen the quantitative outcome results as has been observed by others [4,10].

There is no significant difference in the total counts in the distal nerve between the autograft and tube group at 8 and 16 weeks (Tables 2 and 3). While the number of myelinated axons in the distal segments of the two groups may be similar, the quality of regeneration may be different. For example, in some 8 week mid-tube samples (Fig. 3(C)), NF200+ axons were longitudinally misaligned. From a Toluidine Blue cross-section, one convoluted fibre can be counted multiple times. One way to overcome this is by retrograde labelling of parent motor (located in the lumbar spinal cord enlargement) and sensory (L4-5 dorsal root ganglia) neurons which would prove useful in excluding increased fibre sprouting and branching [24].

## 5. Conclusions

At 4 weeks post-implantation, axonal regeneration was not evident in the PHEMA-MMA tubes, but was

seen in their autograft counterparts. By 8 weeks, similar regeneration was detected in tubes and autografts based on histomorphometry and electrophysiology. *In vivo* results at 16 weeks demonstrated a bimodal response in tubes – 60% showed similar responses to autografts, yet 40% were significantly worse likely due to tube collapse. In ongoing studies, we are designing and creating tubes that will resist collapse in order to have all tubes promote regeneration similar to that of autografts over the long term and over longer gaps, thereby obviating the need for autograft tissue.

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