Video-Gait Analysis of Functional Recovery of Nerve Repaired with Chitosan Nerve Guides

MINAL PATEL, M.S.,1 PAMELA J. VANDEVORD, Ph.D.,1 HOWARD MATTHEW, Ph.D.,2 BIN WU, M.D.,3 STEPHEN DESILVA, M.D.,3 and PAUL H. WOOLEY, Ph.D.1,3

ABSTRACT

Quantitative analysis of peripheral nerve regeneration using nerve guides is commonly evaluated through histomorphometry and walking track analysis. We conducted a unique assessment of functional sciatic nerve recovery treated with chitosan nerve guides. We used video-gait analysis to evaluate the extent of functional nerve recovery by measuring the ankle angle at different gait cycle phases. We also correlated the gastrocnemius muscle weight measurements and histological analysis to functional nerve recovery. The chitosan group showed increased functional improvement compared to the control groups at the end of a 12-week period (p < 0.05). Although both control and chitosan angle measurements were lower than those recorded for presurgery animals, the angle measurements significantly improved over the 12-week period. Stance phase duration of the gait cycle was also recorded, which showed a significant increase over the 12-week time period. The muscle weight parameter indicated a significant decrease in muscle atrophy and restoration of functional strength. Histological analysis revealed that the chitosan nerve guide provided significantly increased axonal growth. The functional results indicated that chitosan nerve guides enhanced functional improvement over no repair processes.

INTRODUCTION

The peripheral nervous system has a very restricted capacity for repair and regeneration in the event of traumatic nerve injury. In most cases, injuries result in gaps, which are reconstructed by microsurgical techniques using autologous nerve grafts. However, autograft utilization has limitations and the repair is usually incomplete, leading to irreversible damage. Additional disadvantages include loss of function at the donor site and the need for multiple surgeries, which increase the risk and cost to the patient. Therefore, implantation of nerve guide tubes is considered an alternative method to achieve nerve regeneration of transected nerves. Commercially, silicone nerve tubes are available for repair of short nerve defects ranging from 1 to 3 cm.1–4 However, full functional nerve recovery is still a challenge even after utilizing the traditional repair method of autografts.5–9 Currently a vast amount of research is being pursued to engineer the ideal nerve guide tube that can promote both sensory and motor function.10–18 Previous studies evaluating sciatic nerve regeneration through nerve guide tubes involved the use of the traditional histological and electrophysiological methods. In addition, it has been reported that quantitative analysis from histomorphometric measurements and electrophysiological recordings lack correlation with functional nerve recovery.19–21 The standard technique for functional evaluation of peripheral nerve regeneration is the walking track analysis.22 However, numerous studies have reported that gait analysis is a more sensitive and accurate method in evaluating functional nerve repair compared to the walking track analysis.23–25 Currently, gait analysis is used in the rat model to evaluate functional nerve recovery after sciatic nerve crush and nerve transection. Only Meek et al. have employed this technique...
in the study of nerve regeneration through nerve guides. They compared biodegradable nerve guides with autologous nerve grafts using video analysis scores.14 Their study investigated only the swing phase measurements from the rat gait cycle and not the stance measurements, which are critical indicators of functional recovery. Thus the “gait” parameter has been widely overlooked when it comes to studying functional nerve regeneration in the rat model. Previous research has shown that chitosan nerve guides have been evaluated using only traditional histomorphometric techniques.15–18 The functional gait analysis technique has not been previously employed for evaluation of chitosan nerve guides.

The current study used video-gait analysis to evaluate functional nerve repair and regeneration through chitosan nerve guides. In addition, histomorphometric measurements and gastrocnemius muscle mass were quantified to evaluate the extent of nerve repair. By utilizing this gait technique, our findings provide a preliminary indication that chitosan can promote functional nerve repair and regeneration.

MATERIALS AND METHODS

Fabrication of chitosan nerve guides

Chitosan powder (80% N-deacetylated, 600,000 molecular weight; Biosyntech, Quebec, Canada) was dissolved in 2% acetic acid to form a 2% chitosan solution that was used to fabricate nerve guides. Fabrication was achieved using the dip coating technique of a cylindrical core as previously described in Matthew et al.26 The final constructs consisted of porous chitosan nerve guides with internal diameter of 1.4 mm, wall thickness of 0.6 mm, and length of 12 mm. The guides were stored in 70% ethanol until implantation, when the guides were equilibrated with sterile distilled water and rinsed in phosphate-buffered saline.

Animals and implantation of nerve guides

Twenty-four female Lewis rats (200–225 g) were divided equally into the chitosan, autograft, and unrepaired groups. During implantation surgery, rats were anesthetized and the left sciatic nerve was exposed under a dissection microscope. A 10 mm gap was created and chitosan nerve guides were used to bridge this gap in the chitosan rats. Both transected proximal and distal nerve stumps were inserted into the nerve guide up to a distance of 1 mm and secured in place by a single 10–0 nylon suture. In the autograft rats, a 1 cm section of the sciatic nerve was removed, reversed, and sutured back in place via a single 10–0 nylon suture. In the unrepaired rats, ½ cm of the sciatic nerve was transected but the gap was not bridged. The implants remained in the animal postoperatively for 12 weeks until the animal was euthanized. All procedures were approved by the Wayne State University Animal Investigation Committee.

Training protocol for rats

Before all surgical procedures, the rats were trained to walk on a motorized treadmill (Northern Arizona University). The treadmill was constructed with narrow lanes separated by Plexiglas walls, consisting of a black box area at one end and an electric shock grid at the other end. A training protocol was devised to train the rats to run on the treadmill within 5 days. Each animal was placed on the treadmill facing the black box end and was allowed to explore the area for a few minutes. The treadmill was not turned on during the exploration period. Initially training sessions began at a speed of 17 cm/s at day 1. This was increased to 21 cm/s at day 2 and day 3 and finally to 30 cm/s at days 4 and 5. Each training session lasted for a period of 20 min/day. It should be noted that during the training session or pre- and postsurgical use, activation of the electric shock grid was not necessary for any of the rats. Once the rats were trained, from the 2nd week onwards to the 12th week, presurgical biweekly gait analysis was performed using digital video recordings of the rats walking on a treadmill at a speed of 21 cm/s and 30 cm/s. Similarly, postsurgically from the 2nd week onwards, gait analysis was performed biweekly until the end of the recovery period of 12 weeks. During each postsurgical gait analysis session the rats were allowed to walk on the treadmill for one period of 20 min/week at 30 cm/s.

Functional gait analysis

Functional analysis was performed using rat gait kinematics,27 which includes the study of ankle angle (foot horizontal angle) by digitally video recording rats walking on a treadmill. The walking patterns of rats both pre- and postsurgery were filmed at 30 frames/s, creating blur-free stills for the analysis of individual footsteps. Walking movements were filmed until steady continuous walking patterns were obtained for each rat from the side (Fig. 1). The video recordings were transferred to a computer using Ulead Video Software (Torrance, CA) and converted to single-frame images. The frames were then evaluated using image analysis software (Image-Pro Plus, Media Cybernetics, San FIG. 1. Digital video recording of a rat’s left hindlimb while walking on a motorized treadmill.
Diego, CA) to measure the postural changes of the rat’s hindlimb over the nerve regeneration period. The maximum angle formed by the horizontal foot, ankle, and vertical toe was measured in degrees at midstance (ms), terminal stance (ts), and midswing (msw) phases (Fig. 2II). The angle is an imaginary line, which passes through the knee to the ankle and from the metatarsal head to the ankle (Fig. 2I). During the course of nerve regeneration, it is therefore possible to observe angle measurement changes from preimplant to postsurgery and during the recovery period. The duration for stance phase for the normal (presurgery) and operated (postsurgery) left hindlimb was measured. These measurements were made from sequential single frame images. The stance phase duration was the amount of time the hindlimb stayed in contact with the ground.

**Histological evaluation of nerve regeneration and muscle weight analysis**

Histological evaluation of chitosan nerve guides in the rats was performed at the end of the recovery period of 12 weeks. The nerve guides including proximal and distal stumps were
excised along with the surrounding tissue. Recovered implants were fixed in 10% buffered formalin, dehydrated, and embedded in paraffin blocks. Five-micron sections were cut across the transverse axis starting from the distal to the proximal end. Similar sections were taken from the autograft and unrepaired groups. The sections were mounted and stained with Glees & Marshland’s Silver Stain and Luxol Fast Blue. Stained sections were examined via light microscopy at ×40 magnification using image analysis software (Image-Pro Plus, Media Cybernetics, San Diego, CA). A systematic random sampling procedure was used for histomorphometric analysis. The total nerve section area was divided into 6 sampling fields (45,000 μm²). Each sampling field from the distal, center, and proximal stained regions was used for data collection and each field was divided into 9 subfields (4500-μm² boxes). The middle subfield (box) was randomly selected for collecting data to avoid over- or underestimation of data values. Myelinated and nonmyelinated axon counts, axon diameter, and myelin thickness measurements were collected from the proximal, center, and distal regions. The ratio of the number of axons to the total subfield area was also calculated to estimate the number of axons/μm². The fiber density, which was the ratio of the total axonal area to the subfield area, was also measured.

Statistical analysis

Statistical analyses were conducted using SPSS (SPSS, Chicago, IL). To evaluate differences between or among groups, analysis of variance (ANOVA) was performed with post hoc pairwise testing, when necessary, using the Scheffé test. An α level of 0.05 was selected for significance for all of the statistical tests. The muscle weight and terminal stance angle value were correlated via linear regression for all data pairs in all groups.

RESULTS

Functional analysis of nerve regeneration

Before surgery, rats were trained to run on a treadmill to allow the left hindlimb to be filmed for gait analysis. The angle measurements were made at midstance, terminal stance, and midswing for presurgical rats. From the 2nd to 4th week postsurgery, the autograft group was unable to place their operated foot on the treadmill belt continuously at both speeds of 21 cm/s and 30 cm/s. The paw would be placed on the belt for one gait cycle phase and for the next continuous few cycles the rat would hold its paw in the air, after which it would again place the paw on the belt for one gait cycle and again repeat the limping characteristics. From the 6th week this characteristic limping was not seen and the rats were able to put weight on the operated paw continuously. For the unrepaired group during the 2nd week postsurgery, 2 of 8 rats could not run continuously for 20 min at both 30 cm/s and 21 cm/s. This continued until the 6th week, after which continuous walking was seen for 20 min. At 21 cm/s similar results were also seen for the chitosan group for 4 out of 8 rats until the 8th week. After the 8th week, continuous walking was seen for 20 min. At 30 cm/s, the chitosan group rats ran continuously with no stops. No signs of automutilation or swelling were seen in any of the groups. Table 1 contains the measurements for the left hindlimbs of the chitosan, autograft, and unrepaired groups at midstance, terminal stance, and midswing phases.

Functional gait analysis

Midstance phase. The midstance angle measurements for normal rats were 50 ± 4 and postsurgery for the chitosan, autograft, and unrepaired groups they were 44 ± 11, 40 ± 11, and 21 ± 10, respectively, at the 12th week (Table 1). During the midstance phase an increase in angle measurement was seen for the chitosan group from the 4th to 12th week. The autograft group shows an increase in angle value from the 2nd to 6th week, after which a decrease is seen until the 12th week, during which the angle value increases. Unrepaired group measurements had no significant change throughout the 2nd to 12th week period. Statistical significance was seen between the chitosan and autograft groups for the midstance phase during the 2nd, 10th, and 12th weeks (p < 0.05). Statistical significance was also seen between the autograft and unrepaired group for the midstance phase during the 12th week (p < 0.05).

### Table 1. Midstance (MS), Terminal Stance (TS), and Midswing (MSW) Angle Measurements in Degrees (Mean ± SD) for Chitosan, Autograft, Unrepaired, and Normal Groups from 2nd to 12th Week Time Points at 30 cm/s

<table>
<thead>
<tr>
<th>Weeks</th>
<th>Chitosan MS</th>
<th>Autograft MS</th>
<th>Unrepaired MS</th>
<th>Normal MS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>37 ± 10*</td>
<td>79 ± 9**</td>
<td>29 ± 3</td>
<td>50 ± 5</td>
</tr>
<tr>
<td>2</td>
<td>31 ± 11</td>
<td>73 ± 9</td>
<td>30 ± 5</td>
<td>46 ± 5</td>
</tr>
<tr>
<td>4</td>
<td>34 ± 10</td>
<td>77 ± 11</td>
<td>35 ± 8</td>
<td>46 ± 5</td>
</tr>
<tr>
<td>6</td>
<td>38 ± 6</td>
<td>81 ± 10</td>
<td>29 ± 4</td>
<td>46 ± 5</td>
</tr>
<tr>
<td>8</td>
<td>37 ± 9*</td>
<td>86 ± 19***</td>
<td>29 ± 7</td>
<td>54 ± 5</td>
</tr>
<tr>
<td>10</td>
<td>44 ± 11*</td>
<td>83 ± 7***</td>
<td>24 ± 2</td>
<td>50 ± 4</td>
</tr>
<tr>
<td>12</td>
<td>37 ± 9</td>
<td>86 ± 19***</td>
<td>29 ± 7</td>
<td>54 ± 5</td>
</tr>
</tbody>
</table>

*p < 0.05 when the chitosan group was compared with the autograft group; **p < 0.05 when the chitosan group was compared with the unrepaired group; ***p < 0.05 when the autograft group was compared with the unrepaired group.
**Terminal stance phase.** The terminal stance angle measurements for normal rats were 98 ± 6 and postsurgery for the chitosan, autograft, and unrepaired groups they were 83 ± 7, 80.4 ± 15, and 68 ± 7, respectively, at the 12th week (Table 1). During the terminal stance phase both autograft and unrepaired groups had a low angle measurement at the 2nd week period compared to the chitosan group. At the 2nd week, statistical significance was noted between the chitosan and unrepaired groups (p < 0.05), but no difference was seen when the chitosan group was compared to the autograft group. After the 4th week, the angle value for the chitosan group exhibited a steady increase that continued up to the 12th week from 73 ± 9 to 83 ± 7. When the chitosan group was compared to the autograft group for the terminal stance phase, statistical significance (p < 0.05) was seen at the 10th and 12th weeks. In the autograft group, an increase in angle value was seen from the 4th to 8th weeks, after which a substantial increase was seen only at the 12th week. Whereas in the unrepaired group the values remain within the average range of 53–69 angle measurements and no significant increase was seen throughout the 12-week recovery period. Statistical significance was seen between the autograft and unrepaired groups only during the 2nd week (p < 0.05). Statistical significance was seen between the chitosan and unrepaired groups during the 2nd, 10th, and 12th weeks. Even though the angle measurements of the experimental rats during terminal stance phase did increase over the 12-week period, they failed to return to normal values.

**Midswing phase.** The midswing angle measurements for normal rats were 20 ± 2 and postsurgery for the chitosan, autograft, and unrepaired groups they were 24 ± 2, 21 ± 7, and 25 ± 7 respectively at the 12th week (Table 1). During the midswing phase, the chitosan group had increased angle measurements up to the 6th week, after which they showed a decline up to the 12th week. The autograft group had an increased value at the 2nd week postsurgery, until the 10th week time point, after which a drop was seen. The unrepaired group did not show a decrease in angle measurement, but remained within the 25–35 angle range, indicating that the contralateral limb still had to compensate for the operated limb. No significant differences were seen between groups at the midswing phase, from the 2nd to 12th week time points. Angle measurements were also recorded at 21 cm/s and no significant difference was seen between groups at any time point for any gait cycle phase.

**Stance phase duration.** The duration of the gait cycle stance phase was measured at both 21 cm/s and 30 cm/s both before and after surgery in all 3 groups. At 21 cm/s, the stance phase duration was not significantly different among all 3 groups. At 30 cm/s, the stance phase duration for the chitosan group was lower than for the autograft, unrepaired, and normal groups during the 2nd, 4th, and 6th weeks (Table 2). At the 8th, 10th, and 12th weeks a significant increase in duration was seen in the chitosan group compared to the autograft and unrepaired group (p < 0.05).

**Macroscopic and histological evaluation of nerve regeneration**

At the 12th week rats were euthanized and the repaired nerve composites were excised from the surrounding tissue. Gross pathology indicated there was no tissue inflammation. At 12 weeks postimplantation, histological analysis revealed fragmentation of the nerve guide; thus complete degradation was not seen.

**Histomorphometric analysis of nonmyelinated axons.** At the end of the 12-week period, the number of axons in the distal, center, and proximal regions of the chitosan group were significantly higher than the unrepaired group (p < 0.05), but a significant difference was not seen when the chitosan group was compared to the autograft group (Fig. 2). On comparison of the autograft group to the unrepaired group, a significant difference was seen in all 3 regions (p < 0.05) (Table 3). Similarly, the number of axons per µm² in the distal, center, and proximal regions of the chitosan group were significantly higher than in the unrepaired group (p < 0.05), but a significant difference was not seen when the chitosan group was compared to the autograft group. On comparison of the autograft group to the unrepaired group, a significant difference was seen in all 3 regions (p < 0.05) (Table 3).

The area of axons per subfield was also calculated to estimate the fiber density percentage. In the distal region, 12% of the subfield area was occupied by axons for the chitosan group as compared to only 6% for the autograft group and 3% for the unrepaired group (Fig. 3). In the center region, 11% of the subfield area was occupied by axons in chitosan group as compared to 7% for the autograft group and 3% for the unrepaired group. In the proximal region 13% of the subfield area was occupied by axons as compared to 6% for the autograft group and 4% for the unrepaired group. The nerve fiber density from the distal to the proximal region was statistically significant in the chitosan group.

**Table 2. Stance Phase Duration Measured in Milliseconds (mean ± SD) for Chitosan, Autograft, Unrepaired, and Normal Groups from 2nd to 4th Week Time Points at 30 cm/s**

<table>
<thead>
<tr>
<th>Weeks</th>
<th>Chitosan</th>
<th>Autograft</th>
<th>Unrepaired</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>115 ± 18*</td>
<td>152 ± 13</td>
<td>132 ± 25</td>
<td>185 ± 30</td>
</tr>
<tr>
<td>4</td>
<td>115 ± 18*</td>
<td>162 ± 42</td>
<td>148 ± 34</td>
<td>181 ± 25</td>
</tr>
<tr>
<td>6</td>
<td>133 ± 19</td>
<td>161 ± 32</td>
<td>137 ± 34</td>
<td>177 ± 41</td>
</tr>
<tr>
<td>8</td>
<td>165 ± 20***</td>
<td>124 ± 36</td>
<td>132 ± 57</td>
<td>188 ± 46</td>
</tr>
<tr>
<td>10</td>
<td>154 ± 17*</td>
<td>116 ± 16</td>
<td>121 ± 17</td>
<td>179 ± 55</td>
</tr>
<tr>
<td>12</td>
<td>154 ± 17***</td>
<td>115 ± 27</td>
<td>91 ± 18</td>
<td>177 ± 57</td>
</tr>
</tbody>
</table>

*p < 0.05 when the chitosan group was compared with the autograft group; **p < 0.05 when the chitosan group was compared with the unrepaired group.
<table>
<thead>
<tr>
<th>Region</th>
<th>Chitosan</th>
<th>Autograft</th>
<th>Unrepaired</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Proximal</td>
<td>Center</td>
<td>Distal</td>
</tr>
<tr>
<td>Axon diameter (μm)</td>
<td>3.6 ± 1.7*</td>
<td>2.9 ± 1.1***</td>
<td>2.9 ± 1.1***</td>
</tr>
<tr>
<td>Axon count</td>
<td>53 ± 24**</td>
<td>56 ± 21**</td>
<td>67 ± 38**</td>
</tr>
<tr>
<td>Number of axons per μm² (×10⁻² μm²)</td>
<td>1.2 ± 6**</td>
<td>1.3 ± 5**</td>
<td>1.41 ± 9**</td>
</tr>
</tbody>
</table>

*p < 0.05 when the chitosan group was compared with the autograft group; **p < 0.05 when the chitosan group was compared with the unrepaired group; ***p < 0.05 when the autograft group was compared with the unrepaired group.

FIG. 3. Photomicrographs of regenerated sciatic nerve sections 12 weeks after surgery. (I) Sections from proximal region of regenerated nerve repaired with chitosan tube (A), autograft (B), and unrepaired (C). (II) Sections from central region of nerve repaired with chitosan tube (D), autograft (E), and unrepaired (F). (III) Sections from distal region of nerve repaired with chitosan tube (G), autograft (H), and unrepaired (I). Glees & Marshland’s silver stain; scale bar = 20 μm.
compared to both autograft and unrepaired groups ($p < 0.05$).

The axon diameter was also measured from the distal, center, and proximal regions for all 3 groups. The axon diameter of chitosan group was significantly higher than that of the autograft group in all 3 regions ($p < 0.05$). For the unrepaired group a significant difference was seen only at the distal and center region ($p < 0.05$). On comparison of the autograft group to the unrepaired group, a significant difference was seen in all 3 regions ($p < 0.05$) (Table 3).

Histomorphometric analysis of myelinated axons. Myelinated axon count was also calculated from all 3 regions. The number of myelinated axons from the center and proximal regions were significantly higher in the chitosan group compared to the autograft and unrepaired groups ($p < 0.05$). In the distal region the number of myelinated axons in the chitosan group was low compared to both autograft and unrepaired groups (Fig. 4). In the proximal region, the autograft group had a significantly higher number compared to the unrepaired group ($p < 0.05$) (Table 4). The number of myelinated axons per $\mu$m$^2$ was significantly higher in the central and proximal regions for the chitosan group compared to the autograft and unrepaired groups ($p < 0.05$). Similarly, the number was significantly higher in all 3 regions when the autograft group was compared the unrepaired group ($p < 0.05$) (Table 4).

The area of myelinated axons per subfield area was also calculated to estimate the myelinated fiber density percentage. In the distal region, 12% of the subfield area was occupied by myelinated axons for the chitosan group as compared to only 4% for the autograft group and 2% for the unrepaired group. In the center region, 7% of the sub-field area was occupied by myelinated axons in chitosan group as compared to 2% for the autograft group and 2% for the unrepaired group. In the proximal region, 8% of subfield area was occupied by myelinated axons in the chitosan group as compared to 3% for the autograft group and 1% for the unrepaired group. The myelinated nerve fiber density from the distal to the proximal region was statistically significant in the chitosan group compared to both autograft and unrepaired groups ($p < 0.05$) (Fig. 5). Myelin thickness was measured for all 3 groups. In the distal region, the myelin thickness measurements for the chitosan and autograft group were comparable. For the unrepaired group these values were lower as compared to the chitosan and autograft group.

### Table 4. Comparison of Axon Diameter, Axon Count, and Number of Myelinated Axons per $\mu$m$^2$ for Chitosan, Autograft, and Unrepaired Groups at Proximal, Center, and Distal Regions (Mean ± SD)

<table>
<thead>
<tr>
<th>Myelinated Axons</th>
<th>Chitosan</th>
<th>Autograft</th>
<th>Unrepaired</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Region</strong></td>
<td>Proximal</td>
<td>Center</td>
<td>Distal</td>
</tr>
<tr>
<td>Myelin thickness (µm)</td>
<td>1.9 ± 0.4*** 0.87 ± 0.4 0.98 ± 0.4</td>
<td>1 ± 0.3 1.3 ± 0.3*** 1.09 ± 0.27</td>
<td>0.84 ± 0.25 0.87 ± 0.3 0.82 ± 0.02</td>
</tr>
<tr>
<td>Axon count</td>
<td>43 ± 24*** 52 ± 72*** 25 ± 17</td>
<td>35 ± 19*** 39 ± 19</td>
<td>34 ± 16</td>
</tr>
<tr>
<td>Number of axons per $\mu$m$^2$ ($\times 10^{-2}$ $\mu$m$^2$)</td>
<td>9.9 ± 5*** 10.3 ± 1.7*** 8.3 ± 4</td>
<td>7.2 ± 4*** 9.1 ± 3***</td>
<td>9.6 ± 3.7*** 5.3 ± 2 4.9 ± 2.4 4.8 ± 3</td>
</tr>
</tbody>
</table>

* $p < 0.05$ when the chitosan group was compared with the autograft group; ** $p < 0.05$ when the chitosan group was compared with the unrepaired group; *** $p < 0.05$ when the autograft group was compared with the unrepaired group.
groups. The myelin thickness for the center region axons was significantly higher in the autograft group than the chitosan and unrepaired groups (p < 0.05). The thickness in the proximal region was significantly higher in the chitosan group compared to both the autograft and unrepaired groups (p < 0.05).

Gastrocnemius muscle. The wet gastrocnemius muscle weight for all 3 groups was also measured postsurgery (Fig. 6). Statistical significance was obtained between chitosan and unrepaired groups, confirming that because of an increase in muscle mass the chitosan group muscle had been reinnervated and fiber diameter has been increased, leading to an increase in cross-sectional area. There was no significant difference between the chitosan and autograft groups; both groups had comparable mean muscle weight values. The muscle weights for the chitosan group correlated with the terminal stance angle measurements (r = 0.662, p = 0.073 and r = 0.612, p = .107).

DISCUSSION

In this study, we used functional techniques employing video-gait analysis, muscle mass changes, and histological assessments of the nerve to evaluate chitosan nerve guides for their ability to promote nerve regeneration. The video-gait analysis technique has been found to be more accurate compared to the traditional sciatic function index (SFI). Meek et al. compared biodegradable nerve guides with autologous nerve grafts using video analysis scores and concluded that the nerve guide group had a better swing phase score compared to the autologous group. However, terminal and stance phase scores were not recorded in their study. This technique has also been used in peripheral nerve crush and sciatic autograft rat models to study peripheral nerve regeneration. In one study, the ankle stance angle was
used to evaluate sciatic nerve recovery after complete transection, and stance angle measurements were compared with the SFI over a period of 16 weeks. The authors concluded that the angle stance measurements were more sensitive in detecting functional nerve recovery as compared to the SFI. Their data also showed a significant correlation between the stance angle measurements and gastrocnemius muscle weight. Similar results were seen in our study; probably because of the condition that during stance phase the limb has to bear the weight of the body. In cases of complete nerve transection, the muscle becomes weakened and the limb has difficulty in bearing the body weight. This also results in a shortened stance phase angle for both chitosan and unrepaired groups. Therefore, the stance phase for the contralateral limb will inevitably be longer, as it attempts to compensate for the loss of stability due to the operated limb.

Terminal stance phase in normal rats has a maximum angle value, but after sciatic nerve injuries, the terminal stance angle value decreases significantly. As recovery occurs, the terminal stance value increases over time. Thus, terminal stance phase is a critical indicator of muscle strength because as axonal reinnervation of the muscle occurs the value increases proportionally. During midswing phase, normal rats have a low angle measurement because the foot is not in contact with the ground, but remains in the air. After sciatic nerve injury the weight of the rat is redistributed to the contralateral uninjured hindlimb. This limb now has to compensate for the weakened muscle in the injured limb. As recovery occurs, there is a change in the midswing angle measurement from maximum to minimum, indicating a transition from postimplant to recovery. Over the recovery period, the muscle strength increases owing to axonal reinnervation and at this stage the contralateral hind limb does not have to compensate for the operated experimental limb, leading to a reduced angle formation during the swing phase.

After complete sciatic nerve transection, denervation of the gastrocnemius muscle also occurs. This will lead to muscle fibrosis depending on the extent of nerve degeneration and ultimately lead to muscle atrophy. The cross-sectional area and mass of the muscle also reduces dramatically. As the muscle is reinnervated with regenerating axons from the distal nerve stump, the fiber diameter increases which leads to an increase in cross-sectional area. Decrease in muscle atrophy can lead to functional improvement, which has been confirmed by our functional gait studies. Duration of the stance phase also has a correlation with muscle atrophy, which has been demonstrated in a gait analysis study using an experimental peripheral vascular disease rat model. The authors suggested that a decrease in the stance phase was due to the muscle atrophy. Gait stance duration has also been studied to evaluate nerve regeneration through biodegradable nerve guides, although no difference was seen between the nerve guide and control group. The gait analysis was conducted when the rats were walking on a Plexiglas runway, so there was no speed control on the velocity of the rat gait. It has been found that the stance phase duration decreases as the walking speed increases and it was concluded that the maximum walking speed for a rat was 53 cm/s and the minimum was 25 cm/s, after which the rat appeared cautious while walking. Similar observations were made in our study at 21 cm/s. Overall, subjecting rats to exercise has been found to have a positive effect on functional nerve recovery after sciatic nerve injury.

Functional nerve recovery is also dependent on the quality of nerve regeneration, which was evaluated by histological analysis. During nerve regeneration, axons sprout from the proximal nerve stump, attempt to cross the tissue gap, and enter the endoneurial tubes of the distal nerve stump. As regeneration proceeds and axons enter the endoneurial tubes, the axon diameter increases. If reinnervation is prolonged owing to misdirectional growth, distal stump atrophy occurs and the axon diameter decreases. Numerous studies by Tanaka et al. have evaluated chitosan tendon nerve guides blended with laminin peptides. In one of their studies they histologically evaluated nerve regeneration through the chitosan laminin peptide tubes. At the end of a 12-week study, they found that the regenerated axon diameter at the distal end was lower in the chitosan laminin peptide group compared to collagen nerve guides and the isograft group. The myelinated fiber density in the chitosan peptide group and isograft group was significantly larger at the distal end. Similarly, the axon area percentage was also significantly larger in the chitosan peptide group. They concluded that chitosan nerve guides are effective in inducing nerve tissue regeneration. In our study, chitosan nerve guides have provided directional guidance to the regenerating axons, leading to an increase in axon diameter. As a result, an increase in axon area was also seen from the proximal to the distal end. Out of the total axonal area, a large amount of area was occupied by myelinated axons in the nerve guide group, indicating successful axonal reformation at the distal nerve stump. Comparatively, in the autograft group the area of myelinated and nonmyelinated axons were lower even though the numbers of axons were higher. This indicates increased axonal sprouting at the proximal end and at the distal end misdirectional connections or slow axonal growth. In the case of slow axonal growth, the more time it takes for the distal stump to be reinnervated, the thicker the endoneurial sheath becomes owing to progressive collagen deposition. This will restrict the axonal diameter and lead to permanent reduction in axonal size and myelin thickness. Even though the numbers of myelinated and nonmyelinated axons in the autograft group are similar in number to the nerve guide group, the overall axonal area is larger in the nerve guide group. Thus, the closed system of the chitosan nerve guides creates a barrier between the regenerating axons and the surrounding environment. This environment promotes the axon extending from the proximal stump to cross the 10-mm gap and remain in direct contact with the distal stump. Even though chitosan nerve guides provide faster axonal growth

FUNCTIONAL RECOVERY OF NERVE REPAIRED WITH CHITOSAN NERVE GUIDES

16–18,40 have evaluated chitosan tendon nerve guides blended with laminin peptides. In one of their studies they histologically evaluated nerve regeneration through the chitosan laminin peptide tubes. At the end of a 12-week study, they found that the regenerated axon diameter at the distal end was lower in the chitosan laminin peptide group compared to collagen nerve guides and the isograft group. The myelinated fiber density in the chitosan peptide group and isograft group was significantly larger at the distal end. Similarly, the axon area percentage was also significantly larger in the chitosan peptide group. They concluded that chitosan nerve guides are effective in inducing nerve tissue regeneration. In our study, chitosan nerve guides have provided directional guidance to the regenerating axons, leading to an increase in axon diameter. As a result, an increase in axon area was also seen from the proximal to the distal end. Out of the total axonal area, a large amount of area was occupied by myelinated axons in the nerve guide group, indicating successful axonal reformation at the distal nerve stump. Comparatively, in the autograft group the area of myelinated and nonmyelinated axons were lower even though the numbers of axons were higher. This indicates increased axonal sprouting at the proximal end and at the distal end misdirectional connections or slow axonal growth. In the case of slow axonal growth, the more time it takes for the distal stump to be reinnervated, the thicker the endoneurial sheath becomes owing to progressive collagen deposition. This will restrict the axonal diameter and lead to permanent reduction in axonal size and myelin thickness. Even though the numbers of myelinated and nonmyelinated axons in the autograft group are similar in number to the nerve guide group, the overall axonal area is larger in the nerve guide group. Thus, the closed system of the chitosan nerve guides creates a barrier between the regenerating axons and the surrounding environment. This environment promotes the axon extending from the proximal stump to cross the 10-mm gap and remain in direct contact with the distal stump. Even though chitosan nerve guides provide faster axonal growth
compared to the autograft group, further information can be obtained by quantifying the reinnervation of the target muscle.

In conclusion, the presence of a nerve guide provides faster axonal growth over the experimentally created gap. Functional improvement was seen as a result of the use of chitosan nerve guides, based on the video-gait analysis, histological analysis, and improvements in gastrocnemius muscle weight.

ACKNOWLEDGMENTS

This study was funded by a grant from the Department of Orthopaedic Surgery, Funds for Medical Research, Wayne State University, Detroit, MI, 48201. The authors would like to acknowledge the excellent technical expertise of Ms. Lois Mayton.

REFERENCES
