

GDNF–chitosan blended nerve guides: a functional study

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Abstract

Nerve guides are currently being fabricated by blending a variety of biomaterials with different proteins. Adding proteins, which can support nerve repair and regeneration, optimizes the biological properties of a nerve guide. In our study we have blended glial cell line-derived nerve growth factor (GDNF) and laminin with chitosan to fabricate GDNF–laminin blended chitosan (GLC) nerve guides. As GDNF is known to provide trophic support to motor neurons, the main objective of this study was assess the functional restoration of an injured sciatic nerve treated with GLC. Functional nerve recovery was evaluated using a video gait-analysis technique. Gastrocnemius muscle weight measurements and sensitivity testing were correlated to functional nerve recovery. Our results indicate an increase in the functional recovery of the GLC group when compared to the unblended chitosan nerve guides. At the end of 12 weeks, GLC nerve guides had comparable functional values to the Laminin-I blended chitosan nerve guides (LC) and autograft groups, which were both significantly higher at the terminal stance phase angle as compared to the unblended chitosan nerve guides. Muscle weights for the GLC group indicated decreased atrophy and restoration of functional strength, compared to the unblended chitosan groups. In addition, behavioural testing demonstrated that the GLC group regained sensation while the control groups displayed no restoration. Thus, the addition of GDNF and laminin to the chitosan nerve guides enhanced both functional and sensory recovery. Copyright © 2007 John Wiley & Sons, Ltd.

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1. Introduction

Many researchers are focusing on blending different proteins with a variety of biomaterials to engineer the ideal nerve guide, in the hope of restoring both sensory and motor function. [1–6] After nerve injury, one of the causes contributing to apoptosis is neurotrophic factor deprivation. [7] Out of these neurotrophic factors, glial cell-line derived neurotrophic factor (GDNF), stands out in providing trophic support to motor neurons. [8,9] GDNF has also been found to rescue and prevent the atrophy of motor neurons. [10] Recently, GDNF has been successfully blended and released from polymer

nerve guides to promote nerve remyelination and repair after nerve injury. A study by Jubran *et al.* (2003) [11] applied fibrin sealant containing GDNF to repair rat sciatic nerve transection. They applied the behavioural hot plate test to evaluate the return of sensory function. Six weeks after surgery, none of the control animals responded to the hot plate test; however, the GDNF fibrin animals had an increased responsiveness. Thus, the results indicated increased sensory recovery after administration of GDNF. Previously, [12] when a polyacrylonitrile/polyvinylchloride nerve guide filled with collagen and GDNF had been applied to repair an 8 mm sciatic nerve gap, increased axonal growth was found within the nerve guide. Fine *et al.* (2002) [13] implanted an ethylene vinyl acetate synthetic nerve guide incorporated with GDNF to bridge a rat sciatic nerve gap for a period of 6.5 weeks.

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After histomorphometric analysis, they concluded that administration of GDNF leads to enhanced motor nerve regeneration. Thus, treatment with GDNF can promote motor axon regeneration.

In general, guidance channels are applied clinically because they form a barrier between the regrowing axons and the surrounding environment, thus limiting scar tissue formation and promoting regeneration. Our study proposed to promote both motor and sensory nerve recovery by utilizing GDNF and laminin-1 blended with chitosan as a material for our peripheral nerve guides. Laminin was chosen as a bioactive component within our nerve guide because it is a natural extracellular matrix protein and a stimulator of axonal outgrowth *in vivo*. [14] The protein contains an active domain for the neurite outgrowth fragment, thus playing an important role in functional nerve recovery. [15] It has also been shown to reduce post-traumatic pain after nerve injury. [16] Chitosan is a cationic natural biopolymer produced by alkaline *N*-deacetylation of chitin. Previous researchers have shown that chitosan is biocompatible and biodegradable, low in cost, due to its abundance, and allows for a diverse range of processing methods. [17,18] In addition, our group has evaluated the use of chitosan alone as a nerve guidance channel in the rat sciatic nerve model. [19] Using gait analysis, we found that chitosan alone significantly increased the functional recovery of rats over controls. However, the results were not comparable to normal values, thus the reason to further modify our nerve guides. Collectively, the components of our novel nerve guidance channel (chitosan, GDNF and laminin-1) have the potential to further enhance both motor and sensory recovery after nerve injury.

Clinically, it is very important to obtain full functional nerve recovery after nerve injury. However, previous published reports have only histologically evaluated the effect of GDNF on motor recovery after injury in animals. Our study evaluated the potential of GDNF–laminin blended chitosan nerve guides to promote functional nerve recovery using gait analysis and muscle mass measurements. We have also studied the effect of our nerve guides on neuropathic pain using mechanical withdrawal threshold testing.

2. Materials and methods

2.1. Fabrication of chitosan nerve guides

Chitosan powder (80% *N*-deacetylated, molecular weight 600 000; Biosyntech, Quebec, Canada) was dissolved in 2% acetic acid to form a 2% chitosan solution. A GDNF–laminin-I blended chitosan nerve guide (GLC) was prepared by dissolving 2 $\mu\text{g}/\mu\text{m}^2$ GDNF (Sigma-Aldrich, St. Louis, MO, USA) and 2 $\mu\text{g}/\mu\text{m}^2$ laminin-1 (Sigma-Aldrich) into the 2% chitosan solution. A laminin-1 blended chitosan nerve guide (LC) was prepared by dissolving 2 $\mu\text{g}/\mu\text{m}^2$ laminin-I into the 2% chitosan

solution. Unblended chitosan nerve guides (C) were also fabricated from the 2% chitosan solution. The guides were stored at 4°C, immersed in 80% ethanol, until implantation, when the guides were equilibrated with sterile distilled water and rinsed in sterile phosphate-buffered saline (PBS).

2.2. *In vitro* GDNF release

Blended chitosan scaffolds (80 μg weight and 5 μg GDNF/scaffold) were incubated in tubes of sterile PBS. Lysozyme (4 mg/ml; Sigma-Aldrich) was added and the tubes were placed in a 37°C incubator–shaker set at 100 r.p.m. Samples and the supernatants were retrieved every 2 days for 14 days. The supernatants were assayed for GDNF concentration using the GDNF Emax ImmunoAssay System Kit (Promega, Madison, WI, USA), as directed by the manufacturer. Optical density was measured at 450 nm, using a microplate reader (Molecular Device Corp., Sunnydale, CA). Three scaffolds were collected at each time point and the experiment was repeated three times. Results were reported as average values \pm standard deviations (SD).

2.3. Animals and implantation of nerve guides

Female Lewis rats ($n = 120$; weight 225–250 g) were divided as follows: experimental GLC nerve guide group; LC nerve guide group; C nerve guide group; unrepaired negative control group; and positive control autograft group. During implantation surgery, the rats were anaesthetized and the left sciatic nerve was exposed under a dissection microscope. A 10 mm gap was created and the nerve guides were used to bridge this gap. Both of the 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 10 mm section of the sciatic nerve was removed, reversed and sutured back in place by a single 10-0 nylon suture. In the unrepaired rats, 5 mm of the sciatic nerve was transected but the gap was not bridged. The implants remained in the animal postoperatively for a period of 6, 9 and 12 weeks until sacrifice. The Wayne State University Animal Investigation Committee approved all procedures.

2.4. Functional gait analysis

Prior to surgery, the rats were trained to run on a treadmill according to a previously described protocol. [19] Briefly, video gait analysis was used to perform functional analysis of rats walking on the treadmill. Digital videos of rat gait kinematics were used to study the angle formed by the knee, ankle and foot. Both pre- and postsurgery, rat walking patterns were filmed at 30 frames/s. Walking movements were filmed from the left side of the treadmill until steady continuous walking patterns were obtained for each rat. Ulead Video Software (Torrance, CA, USA)

was used to transfer the recorded videos to a computer and convert them to single-frame images. Image analysis software (Image-Pro Plus, Media Cybernetics, San Diego, CA, USA) was used to evaluate each frame to measure the postural changes of the rat's hind limb over the nerve regeneration period. Gait cycle phases, including the mid-stance (MS) and terminal stance (TS), were used to measure the angle (in degrees, °) formed by the horizontal foot, ankle and vertical knee. As nerve regeneration proceeded, the angle measurement changes could be observed from pre-implant to postsurgery over the recovery period.

2.5. Sensitivity testing

The sensitivity testing protocol was adapted from Chaplan (1994). [20] Prior to testing, the rats were placed in plastic cages with a wire mesh bottom, allowing full access to the paw. The mid-plantar left hind paw area was tested; avoiding the less sensitive footpads. The paw was touched with one of a series of six von Frey hairs with logarithmically incremental stiffness (2.0, 4.0, 6.0, 8.0, 10.0 and 15.0 g; Stoelting). Each hair was held perpendicular to the plantar surface with sufficient force to cause a slight buckling of the hair and held for 6–8 s. Testing was started with the 2.0 g hair and continued in ascending order. A positive response was noted if the paw was sharply withdrawn. In the absence of a paw withdrawal response to a selected hair, a stronger stimulus was presented in the form of a hair with increasing stiffness. In the presence of a positive response, the next weaker stimulus was chosen. This method determined the 50% paw withdrawal threshold. A total of six responses were noted. The final counts of positive and negative responses were tabulated and scored according to $X = \text{withdrawal}$, $0 = \text{no withdrawal}$, and the 50% threshold response was calculated using the formula:

$$50\% \text{ g threshold} = (10^{[Xf+k\delta]})/10\,000$$

where $Xf = \text{value}$ (in log units) of the final von Frey hair used, $k = \text{tabular value}$ for the pattern of positive/negative responses, and $\delta = \text{mean difference}$ (in log units) between stimuli. Testing was performed prior to surgery and at 12 weeks postsurgery.

2.6. Gastrocnemius muscle weight analysis

The gastrocnemius muscles were isolated and their weights were measured to evaluate axonal re-innervation and restoration of functional muscle strength after 6, 9 and 12 weeks. The lower hind limb gastrocnemius muscle was released from its origin and insertion point. Absolute muscle mass was measured from both the operated and the non-operated contralateral side for all groups. The rat total body weight was also measured for all groups.

2.7. Statistical analysis

Statistical analysis was conducted using SPSS (SPSS Inc., Chicago, IL, USA). To evaluate differences between or among groups, analysis of variance (ANOVA) was performed with *post hoc* pairwise testing, when necessary, using the Scheffé test. A α level of 0.05 was selected for significance for the entire statistical test. The muscle weight values were correlated with MS and TS angle values by using linear regression for all data pairs in all groups.

3. Results

3.1. GDNF–laminin–chitosan and laminin–chitosan blended nerve guides

The nerve guides used in this study had an internal diameter of 1.6 and 0.8 mm wall thickness, as measured with a micrometer. The guides were 12 mm in length (Figure 1). The wall thickness when measured along the length of the tube had an invariable measurement of 0.8 mm. After the fabrication process and implantation, the nerve guides retained their tubular structure and did not collapse, and nerve compression was not noted.

3.2. *In vitro* GDNF release

GDNF release kinetics were monitored for 14 days. After 8 days the cumulative mass of GDNF released from the scaffold reached a peak of 3590 pg/mg, after which the growth factor released was negligible. The accumulated release of GDNF was approximately 70% of the incorporated growth factor after 7 days (Table 1).

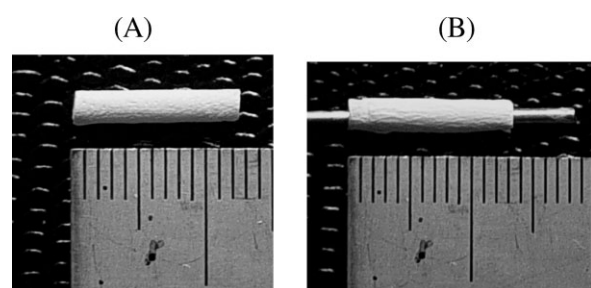


Figure 1. (A) GDNF + laminin + chitosan (GLC) and (B) laminin + chitosan (LC) nerve guides pre-implantation

Table 1. Concentration of GDNF protein release, determined by ELISA technique at 2, 4, 8 and 14 days

Time (days)	Concentration (pg/mg)	SD
2	526	140
4	2485	320
8	3590	105
14	3125	315

3.3. Functional gait analysis

3.3.1. Mid stance (MS) phase angle measurements

After 2 weeks postsurgery, angle measurements for the LC and autograft groups were similar, both being significantly higher than the unrepaired, C and GLC groups ($p < 0.05$). At week 4, the C, LC and autograft group MS angle measurements decreased but were significantly higher than the GLC and unrepaired groups ($p < 0.05$; Table 2). By the 6 week time point, the GLC group angle measurements had significantly increased as compared to the 4 week time point, reaching similar measurements to those of the LC and autograft groups. GLC, LC and autograft groups were all significantly higher than the unrepaired and C groups ($p < 0.05$). At the 10 week time point the angle measurements for the GLC and LC groups were found to be significantly higher than the autograft, C and unrepaired groups ($p < 0.05$). Lastly, at the 12 week time point, the autograft group was significantly higher than unrepaired, GLC and LC groups ($p < 0.05$; Figure 2). No significant

difference was seen for the MS angle phase at the 12 week time point between the GLC and LC groups (Table 2).

3.3.2. Terminal stance (TS) phase angle measurements

At the 2, 4 and 6 week time points the TS phase angle measurement for both the GLC and LC groups were similar to the autograft group, yet significantly higher than the unrepaired and C groups ($p < 0.05$; Table 3). By the 8 week time point, the angle measurements for the GLC and autograft groups were significantly higher than the unrepaired, C and LC groups (Table 3). However, the LC group was only significantly higher than the unrepaired group ($p < 0.05$). At the 10 week time point, angle measurements for the GLC, LC and autograft groups were significantly higher than the unrepaired and C groups ($p < 0.05$; Table 3). Lastly, at the 12 week time point, both the GLC, LC and autograft groups were significantly higher than the unrepaired and C groups ($p < 0.05$; Table 3; Figure 2).

Table 2. Mid-stance (MS) angle (mean \pm SD) for GDNF–laminin–chitosan (GLC), laminin–chitosan (LC), chitosan (C), unrepaired and autograft groups from the 2–12 week time points at 30 cm/s

Weeks	Mid-stance (MS) angle ($^{\circ}$)				
	GLC	LC	C	Autograft	Unrepaired
2	21 \pm 7	29 \pm 13 ^{*,+,+++}	20 \pm 2	29 \pm 6 ^{****,^,^^}	15 \pm 3
4	22 \pm 10	28 \pm 9 ^{*,++}	28 \pm 5 ⁺	28 \pm 13 ^{^,****}	15 \pm 5
6	34 \pm 15 ^{**,**}	29 \pm 10 ⁺	22 \pm 8	32 \pm 10 ^{^,^^}	21 \pm 8
8	31 \pm 13	27 \pm 13	23 \pm 2	30 \pm 13	26 \pm 4
10	36 \pm 14 ^{**,**,****}	35 \pm 9 ^{+,+,++++}	23 \pm 2	20 \pm 4	22 \pm 8
12	29 \pm 4	29 \pm 8	23 \pm 2	40 \pm 11 ^{^,****,+++}	22 \pm 11

* $p < 0.05$ when GLC group compared with LC group.

** $p < 0.05$ when GLC group compared with C group.

*** $p < 0.05$ when GLC group compared with unrepaired group.

**** $p < 0.05$ when GLC compared with autograft group.

+ $p < 0.05$ when LC group compared with C group.

++ $p < 0.05$ when LC group compared with unrepaired group.

+++ $p < 0.05$ when LC group compared with autograft group.

^ $p < 0.05$ when autograft group compared with unrepaired group.

^^ $p < 0.05$ when autograft group compared with C group.

Table 3. Terminal stance (TS) angle (mean \pm SD) for GDNF–laminin–chitosan (GLC), laminin–chitosan (LC), chitosan (C), unrepaired and autograft groups from 2–12 week time points at 30 cm/s

Weeks	Terminal stance (TS) angle ($^{\circ}$)				
	GLC	LC	C	Autograft	Unrepaired
2	64 \pm 9 ^{****,**}	64 \pm 18 ^{+,++}	47 \pm 12	69 \pm 13 ^{^,^^}	26 \pm 6
4	56 \pm 23 ^{****,**}	53 \pm 17 ^{+,++}	40 \pm 4	63 \pm 17 ^{^,^^,+++}	30 \pm 9
6	69 \pm 16 ^{****,**}	68 \pm 20 ^{+,++}	42 \pm 20	72 \pm 10 ^{^,^^}	36 \pm 4
8	75 \pm 17 ^{*,****}	56 \pm 22 ⁺⁺	54 \pm 3	71 \pm 16 ^{^,^^,+++}	35 \pm 9
10	61 \pm 19 ^{****,**}	67 \pm 24 ^{+,++}	43 \pm 2	56 \pm 20 ^{^,^^}	32 \pm 10
12	71 \pm 20 ^{****,**}	69 \pm 17 ^{+,++}	33 \pm 17	80 \pm 15 ^{^,^^}	32 \pm 5

* $p < 0.05$ when GLC group compared with LC group.

** $p < 0.05$ when GLC group compared with C group.

*** $p < 0.05$ when GLC group compared with unrepaired group.

**** $p < 0.05$ when GLC compared with autograft group.

+ $p < 0.05$ when LC group compared with C group.

++ $p < 0.05$ when LC group compared with unrepaired group.

+++ $p < 0.05$ when LC group compared with autograft group.

^ $p < 0.05$ when autograft group compared with unrepaired group.

^^ $p < 0.05$ when autograft group compared with C group.

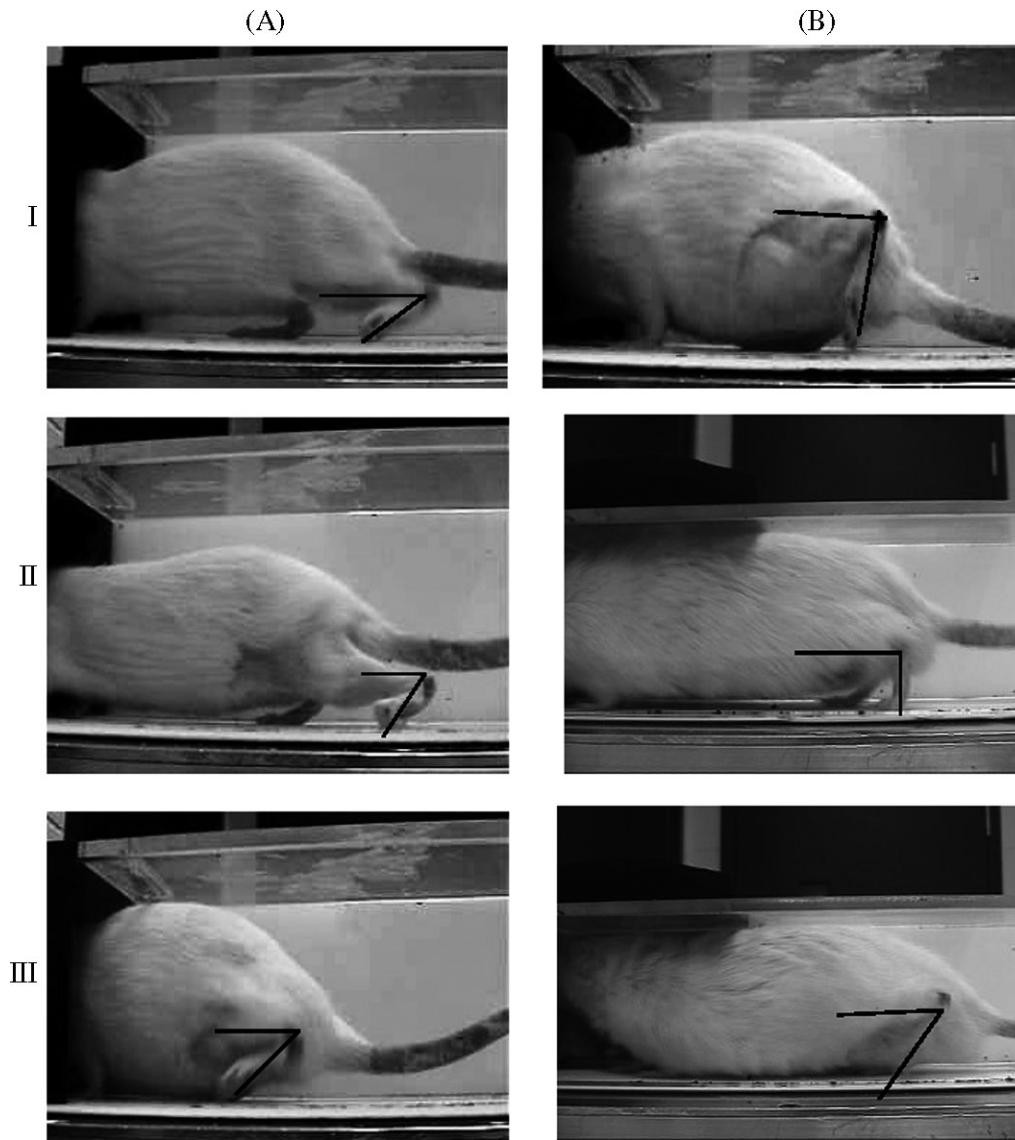


Figure 2. Ankle angle measurements for the GLC (I), autograft (II) and unrepaired (III) groups at 12 weeks, during the mid-stance (A) and terminal stance (B) of the rat gait cycle

3.4. Sensitivity testing

Prior to surgery, the left hind paw had a median withdrawal threshold value of 14.2 ± 0.34 g. The threshold value at 12 weeks dropped to 12.2 ± 1.2 g for the GLC group. Both LC and the unrepaired groups remained constant at the median threshold value of 15.0 g. At 12 weeks the autograft group threshold value dropped to 13.5 ± 0.7 g and the C group had a threshold value of 14.3 ± 0.3 g. Thus, at 12 weeks the withdrawal threshold values for the GLC group were significantly lower than the LC, unrepaired and C groups ($p < 0.05$; Figure 3).

3.5. Gastrocnemius muscle

At 6, 9 and 12 weeks, the gastrocnemius muscle mass measurements for the GLC and LC groups were significantly higher than for the unrepaired group ($p < 0.05$). However, the autograft group measurements were

significantly higher than all the other groups ($p < 0.05$; Figure 4). The total rat body weight was also measured for all groups and no significant difference was noted between groups at all time points; thus, the rat body weight did not contribute to the decrease in overall muscle mass. After 6 weeks, the muscle weight values had a significant correlation between the MS angle values for the GLC group ($r = 0.976$, $p = 0.001$). A significant correlation was not seen for the LC, C, autograft and unrepaired groups. The muscle weight values also had a significant correlation between the TS angle values for both GLC and LC groups ($r = 0.986$, $p = 0.0001$; and $r = 0.920$, $p = 0.009$, respectively). A significant correlation was not seen for the C, autograft and unrepaired groups. After 9 weeks, the muscle weight values had no significant correlation between MS and TS angle values for all groups. After 12 weeks, the muscle weight values had no significant correlation between MS angle values for all groups. The muscle weight values had significant

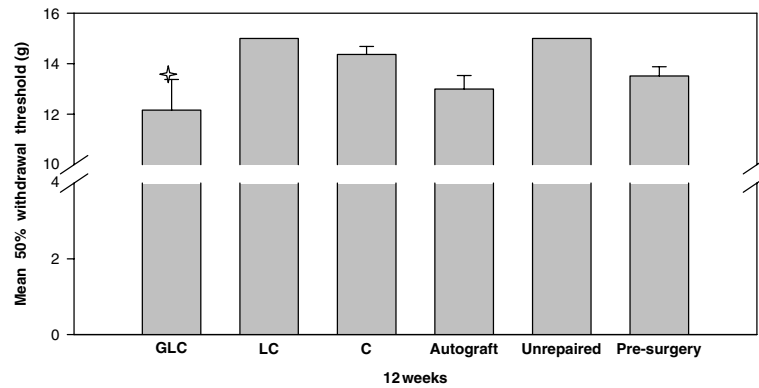


Figure 3. The mean 50% withdrawal threshold of the left hind paw (g) at 12 weeks for the GDNF–laminin–chitosan (GLC), laminin–chitosan (LC), chitosan (C), unrepaired autograft and presurgical groups (mean \pm SE). * $p < 0.05$ for GLC group compared to LC, unrepaired and C groups

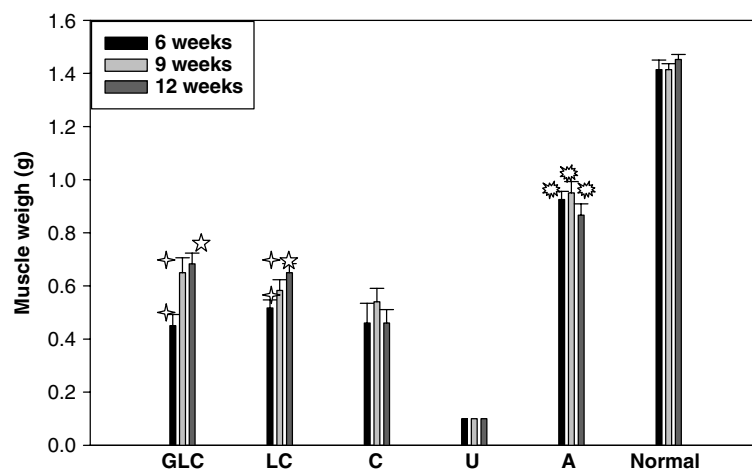


Figure 4. Gastrocnemius muscle wet weights measured for GDNF–Laminin–Chitosan (GLC), Laminin–Chitosan (LC), Chitosan (C), Unrepaired (U), autograft (A) and Normal groups (Mean \pm Std. Error) at the 6th, 9th and 12th week time points. $\star p < 0.05$ when GLC and LC groups compared with unrepaired group, $\star p < 0.05$ when GLC and LC groups compared with unrepaired and Chitosan groups and $\star p < 0.05$ when autograft group compared with remaining groups

correlation between TS angle values for the C group ($r = 0.942$, $p = 0.005$). A significant correlation was not seen for the GLC, LC, autograft and unrepaired groups.

4. Discussion

In this study we evaluated the functional and sensory recovery potential of chitosan nerve guides containing GDNF and laminin, using video gait analysis, muscle mass changes and sensitivity testing. Rat gait techniques have been previously applied to evaluate functional nerve recovery. [19,21,22] A normal gait cycle spans from the ipsilateral limb heel strike, back to the ipsilateral limb heel strike. [23] The MS phase covers 10% of the total gait cycle. During this phase, the ipsilateral foot is in contact with the ground, the ipsilateral heel is in a risen position from the ground and the contralateral foot is in the air. Thus, during this phase the ankle is in a dorsiflexion position and the gastrocnemius muscle is activated. [24] After sciatic nerve transection, the muscle becomes weakened and the ipsilateral foot has difficulty

bearing the rat's body weight. [25] As a result, the ipsilateral foot remains flat on the ground and the heel is unable to rise from the ground. As a result, the ipsilateral foot is unable to support the body weight while the contralateral foot is in the air. This leads to a shortened MS phase angle after nerve transection. However, the angle value increases over time as recovery occurs. In a study by Lin *et al.* (1998), [26] the MS angle measurements were used to evaluate sciatic nerve recovery after complete transection. It was concluded that the MS angle was sensitive in detecting functional nerve recovery and these data showed a significant correlation between stance angle measurements and gastrocnemius muscle weight. In our study the MS angle for the GLC group showed a steady increase until the 10 week time point, indicating gradual recovery. However, at the 12 week time point the angle value was similar to the LC group. As a result, for both GLC and LC groups, the MS angle and muscle weight increased over time, indicating functional improvement.

The TS phase covers 30% of the total gait cycle. During this phase the ipsilateral toes are in contact with the

ground, preparing for toe-off, and the contralateral heel is in contact with the ground at heel strike. During this phase, the ankle is in the dorsiflexion position, the toes in the hyperflexion position and the gastrocnemius muscle is activated. [27] In normal rats the TS phase has a maximum angle value, but after sciatic nerve injury the TS angle value decreases significantly. As recovery occurs, the angle value increases significantly over time. This causes the ipsilateral foot to improve its shock-absorbing properties while the body weight is transferred from the ipsilateral foot to the ipsilateral toe. In our study for the LC group, the TS angle value was not significantly different at the 2 and 12 week time points, whereas for the GLC group the angle value increased significantly between the 2 and 12 week time points, indicating increased muscle strength. However at the 12 week time point there was no significant difference in angle value between the GLC and LC groups. Therefore, in our study the addition of GDNF did not improve motor recovery at the end of the 12 week time point. Previous studies have confirmed that survival of motor neurons by GDNF occurs in a dose- and time-dependent manner. Recently, the treatment of spinal root evulsion by GDNF was evaluated. [28] The dose of GDNF used in this study was 5 µg/µl. GDNF treatment administered 2 weeks after root invulsion injury promoted motor neuron survival, which had a positive effect up to a period of 6 weeks. However, treatment administered 4 weeks postinjury did not promote motor neuron survival. It was concluded that injured adult rat motor neurons die within a few weeks following root invulsion injury, and GDNF treatment after 4 weeks was not effective. These results suggest that optimum motor nerve recovery occurs when GDNF treatment is administered within 2 weeks after injury. Our *in vitro* data demonstrate that the majority of GDNF is released within the first week of incubation. If this translates to the animal model, the amount and time frame of GDNF being released might not be optimal, thus further studies need to be conducted to estimate *in vivo* release.

Von Frey sensitivity testing is routinely used to quantify the degree of neuropathic pain in various sciatic nerve injury models, such as the chronic constriction injury, the spinal nerve ligation and the partial sciatic ligation models. [29,30] After an intensive literature search, we have confirmed that no study has applied von Frey testing to evaluate sensory nerve regeneration through nerve guides. Moreover, only one previous study has applied the von Frey test to evaluate the complete sciatic nerve transection model. [31] In this study, different rat peripheral nerve injury models were studied, using different stimulus tests, including the von Frey testing method. The testing was conducted until 8 weeks and the stimuli ranged from 0 to 80 g. The results suggested that the mechanical threshold value reduced on day 1 from 59.40 to 18.73 g, after which the median value of 18.73 g remained constant until the 8 week time point. Comparatively, our study measured the withdrawal threshold values at 0 and 12 weeks. At 0 weeks, the rats did not respond to any stimuli.

At 12 weeks no change in response was noted for the LC, C and unrepaired groups. Previous studies have shown that, after complete sciatic nerve transection, a zero degree of pain is displayed, compared to partial sciatic transection, which displays a higher degree of pain. [32–34] As axonal connections are re-established, sensory nerve regeneration increases and decreased resistance to applied force is displayed. Thus, this typical increased sensitivity was not seen for the LC, C and unrepaired groups, even after 12 weeks, indicating decreased axonal connections. It should be noted that these groups may be able to feel pain even after the applied stimulus, but due to decreased axonal connections and decreased muscle strength they are unable to lift the foot in response to the stimulus. However, for the GLC group at 12 weeks a decreased resistance to applied stimuli and increased sensitivity was observed. This indicates that axonal connections were re-established through the nerve guide, leading to an increase in muscle strength and sensory nerve regeneration. Previous studies have confirmed that GDNF has a strong effect on sensory nerve regeneration. [35–37] Moreover, studies have also revealed that during the initial nerve injury state GDNF has an analgesic effect on neuropathic pain. [38–40] In conclusion, we have shown that the addition of GDNF to our chitosan nerve guides promotes sensory nerve recovery. Motor nerve recovery utilizing the GDNF–chitosan blended nerve guides resulted in a similar outcome compared to the laminin and chitosan unblended nerve guides.

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