

ORIGINAL ARTICLE

Transplantation of porous tubes following spinal cord transection improves hindlimb function in the rat

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Study Design: Experimental.

Objective: To determine the effects of a porous tube transplant in spinal cord transected rats.

Setting: Acadia University, Wolfville, Nova Scotia, Canada.

Methods: Female rats were randomly assigned to three experimental groups: control (Con, $n=8$), spinal cord transected (Tx, $n=5$) and spinal cord transected with transplant (TxTp, $n=7$). The rats in the TxTp and Tx groups received a complete spinal cord transection at the T10 level and the TxTp group immediately received a porous tube transplant.

Results: Locomotor activity rated on the Basso, Beattie, Bresnahan scale improved significantly in the TxTp animals over the 4 weeks such that final scores were 21, 1.4 and 7.1 for the Con, Tx and TxTp groups, respectively. As expected, the muscle to body mass ratios of the hindlimb skeletal muscles of the Tx group were decreased (soleus 35%, plantaris 29% and gastrocnemius 29%) and this was also observed in the TxTp group (soleus 33%, plantaris 23% and gastrocnemius 30%). Cytochrome c oxidase (CYTOX) activity in the plantaris was decreased by Tx but maintained in the TxTp group (Con = 82.2, Tx = 44.8 and TxTp = 72.8 U/min/g).

Conclusion: Four weeks after the spinal cord transection, plantaris CYTOX activity and locomotor function improved with porous tube implantation.

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Introduction

The effects of a spinal cord injury can be devastating and permanent. These effects include a loss of motor control and sensation in tissues innervated by neurons at or inferior to the level of injury, muscle atrophy and a shift in muscle phenotype.^{1–6} Effective intervention strategies to minimize the loss of muscle mass and to preserve function following spinal cord injury are necessary. One potential approach is the use of biomaterials as implants to create a protective environment in which axons can regenerate following a spinal cord injury. A porous tube composed of a hydrogel biomaterial serves as a nerve guidance channel and may result in decreased scar tissue and increased neural tissue regeneration, thereby overcoming some of the effects of a spinal cord injury.^{7–9}

Creating a protective environment for neuronal growth following a spinal cord injury is essential. Cellular damage to

the cord resulting in myelin debris and scar tissue has been shown to impede locomotor recovery significantly.^{10–13} The amount and type of preserved tissue following a spinal cord injury is highly correlated to recovery and function.^{1,14–16} Small amounts of preserved spinal cord tissue can facilitate large amounts of recovery and locomotor behavior. The Basso, Beattie, Bresnahan (BBB) scale rates the locomotor function by analyzing the movements of the different joints in the hindlimbs of rats. The BBB scale is a validated tool to assess rat locomotion following a spinal cord injury.¹⁷

Following a spinal cord injury, it is well documented that the muscles located below the level of injury experience a decrease in mechanical load and neuromuscular activation. This results in changes of physical and metabolic characteristics of the muscles.^{2–5,18,19} Muscle atrophy and the decrease in muscle size leads to a decrease in force production and development of secondary complications.²⁰ Not only do the muscles decrease in size, but they experience a change in their muscle fiber types characterized by their myosin heavy chain composition. Spinal cord transection in the rat leads to a shift in fiber type to a faster, more fatigable profile, which is associated with a decrease in oxidative potential.

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Cytochrome *c* oxidase (CYTOX) is a commonly used marker for muscle oxidative potential and it has been shown to be sensitive to very early changes in muscle properties following spinal cord transection.²¹ The purpose of this study was to determine the effects of a porous tube transplant following a complete spinal cord transection at the T10 level in rats on locomotor function, CYTOX activity and muscle mass.

Methods

Animal care

This project was approved by Acadia University's Animal Care Committee and all procedures were performed in accordance with the Canadian Council on Animal Care's Guide to the Care and Use of Experimental Animals.²² Adult female Sprague–Dawley rats (Charles River, St Constant, Québec, Canada) approximately 250 g were housed 2–3 per cage. They were fed Pro-lab RMH 3000 pellet food and water *ad libitum*. The rats were given pine chew sticks and slices of raw apple twice a week. Every morning the rats were weighed on an electronic balance (Scout) to record body mass and to monitor health. The rats were randomly assigned to three experimental groups; control (Con, $n=8$), spinal cord transected (Tx, $n=5$) and spinal cord transected with porous tube transplant (TxTp, $n=7$) and studied over a 4-week intervention period. Animals assigned to the control group did not undergo surgery. Thomas *et al.*²³ have shown that locomotor function measured using the 21-point BBB scale was 21 in a sham-operated group at 6 weeks.

Spinal cord transection

Surgical procedures were performed under aseptic conditions following methods described previously.⁴ Briefly, rats were anesthetized with intramuscular (i.m.) injections of Ketaset (ketamine, 60 mg/kg) and Rompun (xylazine, 10 mg/kg). Backs were shaved and cleaned with ethanol (70%) and then an Alphadine solution (10% povidone iodine preoperative preparation) was applied for aseptic cleaning. A 5 cm incision was made longitudinally through the skin. The back muscles were detached from the thoracic vertebrae, and a small retractor was used. A laminectomy was performed to expose the spinal cord. A longitudinal incision was made through the meninges and several drops of Lidocaine (xylocaine hydrochloride, 20 mg/ml) were applied to the spinal cord tissue before cauterizing the dorsal vein caudally and then rostrally. A vacuum pump was used to aspirate a 2 mm section of spinal cord tissue creating a complete spinal cord transection at T10 cord level. Visual inspection of the lesion site was performed to ensure a complete spinal cord transection was performed. Gelfoam (Pharmacia & Upjohn, Kalamazoo, MI, USA), soaked in sterile saline was inserted into the site to promote hemostasis. The Gelfoam was removed and the meninges were sutured with 10-0 silk, and the muscles of the back were sutured with 3-0 silk. The skin incision was closed with wound clips. Warmed dextrose saline solution (5%) and torbugesic (butorphanol, 2 mg/kg) were injected intraperitoneally (i.p.) following surgery. The

rats were placed inside an incubator with a high oxygen concentration on a thermal pad until they regained consciousness.

Porous tubes transplantation

For TxTp rats, a polymeric hollow fiber membrane transplant (porous tube) was implanted immediately following spinal cord transection. As previously described, the porous tubes were synthesized by a novel centrifugal casting process,^{24,25} resulting in porous poly(2-hydroxyethyl methacrylate-co-methyl methacrylate) tubes.^{24,25} These hydrogel tubes were cut into 6 mm lengths and purified overnight in a Soxhelt extractor, individually packaged into cryovials with a punctured cap, and sterilized by autoclaving (20 min at 120°C) before characterization and implantation. The gross physical properties of the channels were soft and flexible, similar in feel to those of contact lenses. The tubes were pulled in tension by a micromechanical tester at a rate of 1%/min, and changes in the load and distance measured.²⁵ The elastic modulus was calculated from the linear portion of the stress–strain curve and represents the elastic behavior or rigidity of the tubes. Tubes had a mean elastic modulus of 311 ± 57 kPa (mean \pm s.d.; $n=4$), and were calculated according to the methods by Dalton *et al.*²⁵

Before implantation, tubes were removed from their sterile vials and transferred into a Petri dish containing a sterile saline solution. The porous tubes were cut to fit the lesion site (approximately 2 mm long) to ensure a flush-fit with the rostral and caudal ends of the spinal cord. Once the porous tube was situated inside the lesion, tisseel (Baxter, Mississauga, ON, Canada) was applied to the ends of the spinal cord and porous tube as an adhesive to ensure that the ends of the tubes remained attached to the spinal cord and the meninges were sutured with 10-0 silk. The rest of the surgery and treatment followed the same methods as the spinal cord transected group.

Care for the animals

After the spinal cord surgery, urinary bladders were manually expressed 2–3 times daily until the voiding reflex returned. If a rat showed signs of infection of the genital area, a topical antibiotic (Polysporin) was applied to the external genitalia. If a urinary tract infection was suspected, or if this treatment was not sufficient, general antibiotics (Baytril 0.05 ml (i.m.) or Duplocillin 0.25 ml (s.c.)) were injected subcutaneously or intramuscularly.

Locomotor assessment

Locomotor assessment was performed using the standardized methods of Basso *et al.*¹⁷ To rate accurately the locomotor ability of the rats in an open-field environment, an empty wading pool and digital video camera were used. Briefly, the rats were videotaped individually for 3–5 min in the open-field environment weekly at approximately the same time of day. If the rats were sedentary while being filmed, they were moved back in the middle of the pool or stimulated to move. Two independent observers, who were

blinded to the treatment condition, watched the videos and scored each rat's locomotor performance individually in accordance with the BBB scale.¹⁷ When the ratings differed, a third observer was used and the average of the scores was taken.

Muscle tissue sampling and analyses

After 4 weeks of intervention, the rats were anesthetized with an injection of Nembutol (sodium pentobarbital, 70 mg/kg i.p.). The rats were weighed and then skeletal muscle tissues including the soleus, plantaris, gastrocnemius and the triceps brachii muscles were dissected. The muscles were quickly weighed, and plantaris muscles were individually wrapped in a foil and snap-frozen in liquid nitrogen. They were stored at -80°C until further analysis.

CYTOX activity of the plantaris muscle was measured in homogenates of individual muscles in triplicate, according to the methods of Smith.²⁶ Briefly, the muscles were homogenized on ice using a polytron homogenizer and 50 μl of the sample was added to the buffer and stock solution of CYTOX. Each sample was reacted for 3 min in a spectrophotometer at 550 nm to quantify the activity of the enzyme and then saturated with potassium ferricyanide to reduce the remaining cytochrome *c*.

Spinal cord tissue sampling and histology

The spinal cords were removed from ~ 1 cm rostral and ~ 1 cm caudal to the lesion site. They were stored in Nalgene vials with approximately 10 ml of Bouin's General Purpose Fixative for a period of 24 h. The spinal cords were then immersed in 70% ethanol for several days and subsequently transferred into glass jars containing 85% ethanol for 2–4 h, 95% ethanol for 1 h and 100% ethanol for 30 min. Finally, they were submersed in toluene for a 1 h period, repeated twice. The spinal cords were infiltrated with paraffin in a vacuum three times for 20 min each. Individual boats were filled with hot paraffin and the spinal cords were embedded in them. A flamed rod was run by the sides of the cords to ensure that no air bubbles were present in the paraffin. The paraffin-filled boats were then set aside to cool and harden. Blocked spinal cords were serially sectioned (10 μm) using an ultramicrotome and mounted on slides. Sections were stained with hematoxylin and eosin and slides were cover-slipped.

Statistical analyses

A repeated measures analysis of variance (ANOVA) was performed on the daily body mass and the BBB scores. A one-way ANOVA was performed for each of the other variables under investigation (Statistica, StatSoft Inc., Tulsa, OK, USA). Tukey HSD *post hoc* tests were performed when statistical differences (accepted at $P < 0.05$) were observed.

Results

A significant decrease in body weight was observed in groups that received the spinal cord transection surgery (i.e. Tx and

TxTp; Figure 1). There was an abrupt drop in weight following the surgery. However, approximately 1 week following the surgeries, the Tx and TxTp groups started to regain weight. The spinal cord transected groups regained weight at approximately the same rate as the control group for the remainder of the intervention period (Figure 1). Despite the weight gain, the transected groups remained significantly lighter than the control animals.

The BBB scores of the three groups are presented in Figure 2. As expected, the Con group scored a perfect 21 at the end of the 4 week intervention. The Tx group experienced a significant drop in locomotor activity compared to the Con group immediately following the spinal cord transection and did not improve significantly in the following weeks (they averaged 1.4 on the BBB scale after 4 weeks). The TxTp group also experienced a significant decrease in locomotor activity following the spinal cord transection surgery. However, after 4 weeks the TxTp group had significantly improved averaging 7.1 on the BBB scale as compared to the Tx group (Figure 2).

CYTOX activity was measured as a marker of oxidative potential in the plantaris muscle 4 weeks following spinal cord transection (Figure 3). Only the Tx group was significantly different from the Con group, being 45.5% lower. The transplant appeared to maintain or restore the oxidative potential of the plantaris muscle since it was not significantly different from controls at the 4-week time point.

Relative muscle weights were calculated following the 4-week treatment period. In the spinal cord transected groups, the muscles of the hindlimb experienced a significant decrease in relative weight, ranging from 28.5 to 35.3% in the Tx group and 23.3 to 33.3% in the TxTp group. The soleus muscle (Figure 4a) experienced the largest decrease in relative muscle weight at 4 weeks. In contrast to the

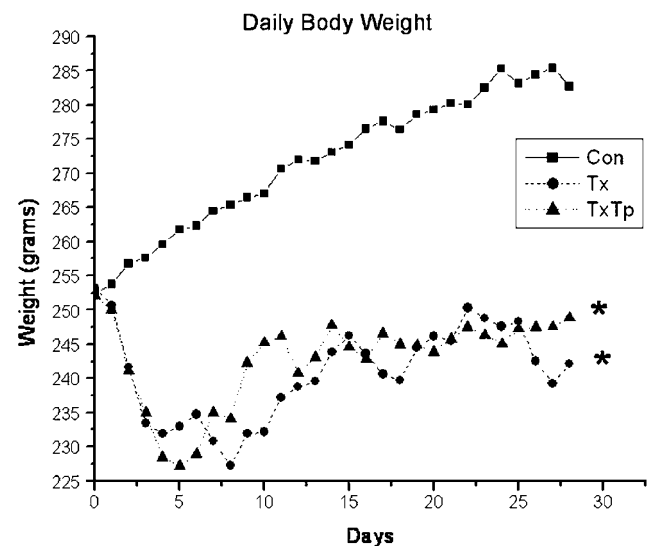


Figure 1 Average daily body mass over time for control (Con, $n=8$), spinal cord transected (Tx, $n=5$) and spinal cord transected with porous tube transplant (TxTp, $n=7$) groups. Group means are plotted. *Significant ($P < 0.05$) decrease compared to control.

hindlimb skeletal muscles, the triceps brachii muscle (Figure 4d) was not significantly affected by the effects of the spinal cord transection. Similar findings were observed for absolute muscle masses (data not shown).

Visual inspections of the spinal cord injury site as well as histological examination of the area confirmed a complete spinal cord transection in the Tx group. Scar tissue was evident as a large deposit of collagen bundles that bridged

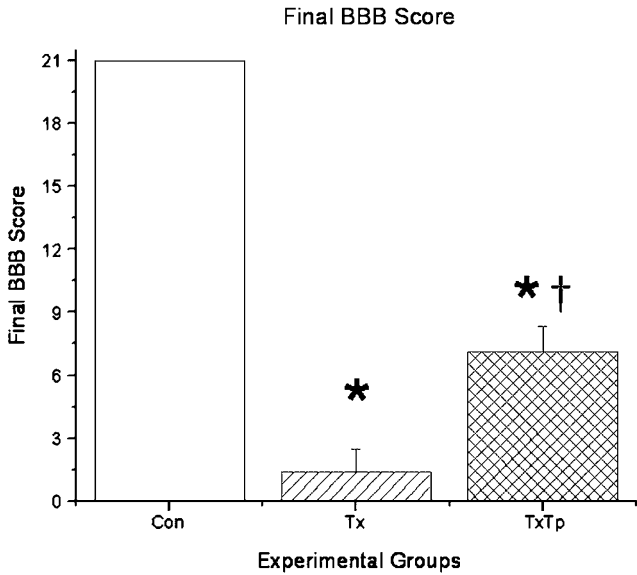


Figure 2 Final BBB scores for control (Con), spinal cord transected (Tx) and spinal cord transected with porous tube transplant (TxTp). Group means ± s.d. *Significant ($P < 0.05$) decrease compared to control. †Significant increase ($P < 0.05$) compared to spinal cord transected (Tx) group.

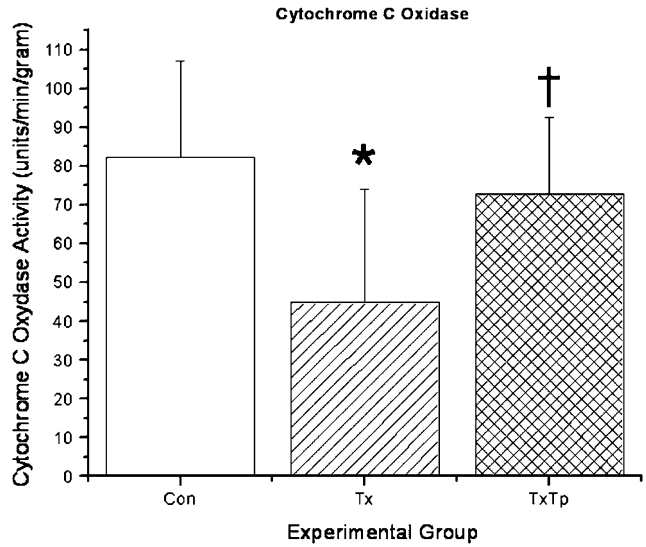


Figure 3 CYTOX activity in plantaris muscle for control (Con), spinal cord transected (Tx) and spinal cord transected with porous tube transplant (TxTp). Group mean activity plotted ± s.d. *Significant decrease compared to control ($P < 0.05$). †Significant increase ($P < 0.05$) compared to spinal cord transected (Tx) group.

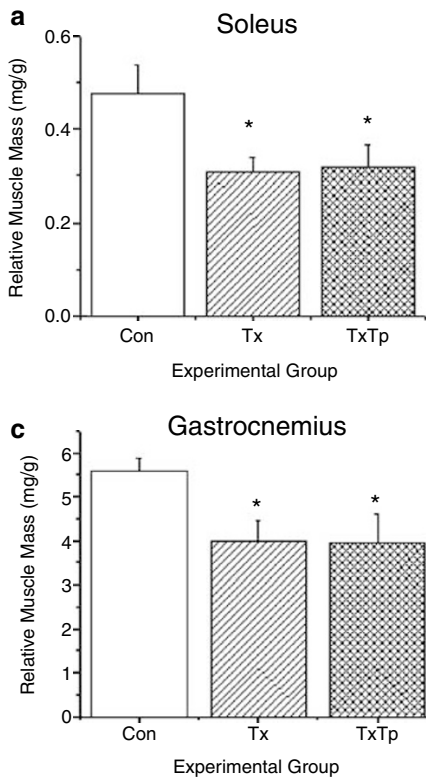


Figure 4 Group mean soleus (a), plantaris (b), gastrocnemius (c) and triceps brachii (d) muscle mass relative to body mass for control (Con), spinal cord transected (Tx) and spinal cord transected with porous tube transplant (TxTp). Group means ± s.d. *Significant decrease compared to control ($P < 0.05$).

the transection area (Figure 5b). In the TxTp group, there appeared to be less scar tissue and in addition, growth of neural tissue was evident at the same level as the transplant, joining the rostral and caudal ends of the spinal cord at the injury site (Figure 5c).

Discussion

The purpose of this study was to determine the effects of the surgical implantation of a porous tube into the lesion cavity following a complete spinal cord transection on the weight and oxidative potential of muscles, and locomotor activity. The results of this study suggest that using a biomaterial transplant following a spinal cord transection can have positive effects on hindlimb skeletal muscle oxidative potential and on locomotion.

The most significant finding of this study was that following the spinal cord transection and transplantation, the BBB scores showed a significant increase in locomotor behavior compared to the spinal cord transection group. The Tx group finished with a mean BBB score of 1.4 and these results are consistent with other spinal cord transection studies.^{12,27,28} The TxTp group finished with a mean score of 7.1 after 4 weeks, and was significantly greater than the Tx group.

The main differences in locomotor behavior between the two groups was that the TxTp group showed extensive movement of all three joints of the hindlimbs (ankle, knee and hip) compared to slight movement of one or two joints in the Tx group. The Tx group averaged a score between 1 and 2 representing only slight movement of two joints or extensive movement of one joint. Although the BBB scores between the Tx and the TxTp groups were statistically significant, neither group displayed significant weight-bearing. These results differ from a recent study⁷ where significant increases in BBB scores with the transplant and neurotrophic factors were observed only after a 7 or 8 week intervention. In the present study, the level of spinal cord transection was lower (T10 vs T8) and the length of the lesion was shorter (~2 vs ~4 mm) thus potentially influencing the rate of recovery. Another explanation for the difference in BBB scores is smaller/younger (~250 vs 200–500 g) female rats were used. It has been shown that younger animals have greater recovery potential following central nervous system injuries including spinal cord lesions.²⁹ The precise reasons for these differences in BBB scores between spinal cord transected and transplanted groups may be due to the different levels of spinal cord tissue regeneration and varying degrees of scar tissue formation. Tissue sparing and tissue regeneration have previously been associated with an increase in locomotor activity.^{1,14–16} Longer intervention periods and the addition of neurotrophic factors may also influence functional recovery.

The spinal cords were sectioned and stained to confirm completeness of the spinal cord transection lesion and to attempt to provide possible mechanisms for the observed changes in skeletal muscle. There was apparent re-establishment of connection of rostral and caudal ends of the spinal cord in the TxTp animals as shown in Figure 5. Reconnection

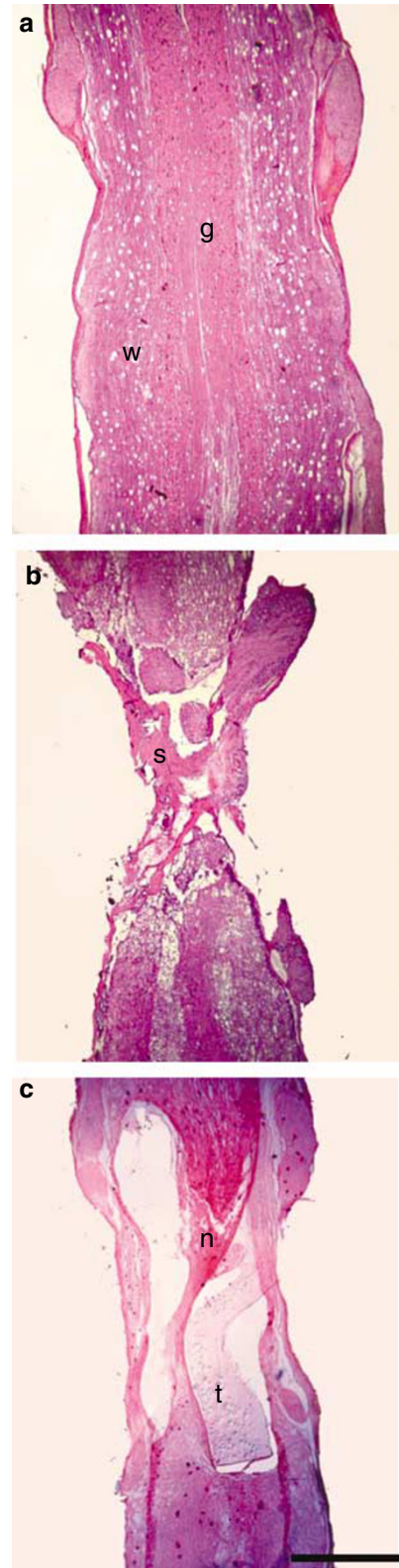


Figure 5 Photomicrographs of longitudinally sectioned spinal cord tissue at the T10 level. (a) Control (Con), (b) spinal cord transected (Tx), (c) spinal cord transected with porous tube transplant (TxTp), w = white matter, g = gray matter, s = scar tissue, n = new neural tissue, t = transplant. Calibration bar, 1 mm.

across the lesion cavity was not observed in any animal in the Tx group. It is well documented that following a spinal cord transection or injury, the development of scar tissue is correlated with a decrease in axonal growth and locomotor recovery.^{15,17,30}

It is accepted that following a spinal cord transection surgery, the muscle fiber type shifts to a faster, more glycolytic phenotype. This shift would also be reflected in the metabolic characteristics of the muscle. In a denervation study conducted by Wick and Hood,³¹ they reported that as early as 8 days after denervation, CYTOX activity was significantly decreased from control levels. The present study demonstrated that a complete spinal cord transection significantly reduces the CYTOX activity in the plantaris muscle. This was expected as the muscle fiber types were shifting toward a more glycolytic phenotype. The porous tube transplant prevented these effects on CYTOX activity, suggesting that this intervention can prevent some of the changes in skeletal muscle fibers following spinal cord transection. Possible mechanisms for the maintained CYTOX activity in the plantaris could be reconnection of neural tissue at the lesion site, the increased activity (electrical or mechanical) of the skeletal muscle, a reduction of central scar tissue or some combination of the three.

Following complete spinal cord transection, there was a significant drop in relative muscle masses of the hindlimb skeletal muscles studied compared with control animals. The use of a porous tube transplant for a 4-week intervention period did not reduce or reverse the amount of muscle atrophy. In this study, approximately 35% of the soleus muscle mass relative to body weight was lost at 4 weeks. Previous studies have reported similar decreases. For example, 15 and 30 days following a spinal cord transection, the soleus muscle mass was approximately 30% smaller than control values.^{4,6} Since the soleus muscle is predominantly comprised of slow, type I muscle fibres, it is particularly susceptible to muscle atrophy, and muscle phenotype changes.⁵

Two other hindlimb muscles, the plantaris and gastrocnemius also demonstrated significant decreases in muscle mass to body mass ratios (plantaris 28.8%; gastrocnemius 28.5%) following spinal cord transection. The relative muscle mass of the transplant group and the transected group were similar in these faster ankle extensors. The triceps brachii muscle mass to body mass ratio was the same in all the three groups, demonstrating that muscle atrophy is only observed in the muscles innervated by neurons at or below the level of injury.

It is possible that a longer intervention with porous tubes may decrease the amount of atrophy since the BBB scores of the TxTp group were significantly higher than the Tx group at 4 weeks. With an increase in locomotor activity, it is expected that the electrical activity and mechanical load on the muscles would be increased and would contribute to altering muscle properties. Perhaps the loading and activity at a score of 7.4 on the BBB scale were insufficient, or the duration of the intervention was too short to restore muscle mass.

Following the surgeries there was an immediate decrease in body mass of the two spinal cord injured groups. The

initial drop in body weight was likely due to the invasiveness of the surgery. After approximately 1 week of recovery, the spinal cord transected groups began to gain weight. The initial loss of body mass following surgery is consistent with other spinal cord transection studies in rats.^{6,32} Because of this change in body mass, the use of a muscle to body mass ratio is preferred although the same trends were observed with absolute muscle masses.

A limitation of this study is that the histology was not sufficient to fully address the question of axonal regeneration; a reduction in scar tissue appears to be a more plausible mechanism to explain the observed results. The improvements in locomotion and oxidative enzyme activity warrant future studies that could include a neurofilament stain to show axons extending across the lesion, or with tracing methods. Also, a stain for glial fibrillary acidic protein to demonstrate a difference in scarring could be useful.

Conclusions

The results of this study demonstrate that transplantation of a porous tube following a spinal cord transection surgery significantly improves locomotor function after 28 days in adult rats. The use of a porous tube also maintains CYTOX activity in the plantaris muscle. Future work is needed to assess the mechanisms responsible for these observed changes in muscle properties and function.

Acknowledgements

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References

- Bresnahan JC, Beattie MS, Todd III FD, Noyes DH. A behavioral and anatomical analysis of spinal cord injury produced by a feedback-controlled impaction device. *Exp Neurol* 1987; **95**: 548–570.
- Dupont-Versteegden EE, Houle JD, Gurley CM, Peterson CA. Early changes in muscle fiber size and gene expression in response to spinal cord transection and exercise. *Am J Physiol* 1998; **275**: C1124–C1133.
- Lieber RL. Skeletal muscle adaptability. II: Muscle properties following spinal cord injury. *Dev Med Child Neurol* 1986; **28**: 533–542.
- Murphy RJL, Dupont-Versteegden EE, Peterson CA, Houle JD. Two experimental strategies to restore muscle mass in adult rats following spinal cord injury. *Neurorehabil Neural Repair* 1998; **13**: 125–134.
- Otis JS, Roy RR, Edgerton VR, Talmadge RJ. Adaptations in metabolic capacity of rat soleus after paralysis. *J Appl Physiol* 2004; **96**: 584–596.
- Talmadge RJ, Roy RR, Caiozzo VJ, Edgerton VR. Mechanical properties of rat soleus after long-term spinal cord transection. *J Appl Physiol* 2002; **93**: 1487–1497.
- Tsai EC, Dalton PD, Shoichet MS, Tator CH. Matrix inclusion within synthetic hydrogel guidance channels improves specific supraspinal and local axonal regeneration after complete spinal cord transection. *Biomaterials* 2006; **27**: 519–533.

- 8 Katayama Y, Montenegro R, Freier T, Midha R, Belkas JS, Shoichet MS. Coil-reinforced hydrogel tubes promote nerve regeneration equivalent to that of nerve autografts. *Biomaterials* 2006; **27**: 505–518.
- 9 Tsai EC, Dalton PD, Shoichet MS, Tator CH. Synthetic hydrogel guidance channels facilitate regeneration of adult rat brainstem motor axons after complete spinal cord transection. *J Neurotrauma* 2004; **21**: 789–804.
- 10 Fawcett JW, Asher RA. The glial scar and central nervous system repair. *Brain Res Bull* 1999; **49**: 377–391.
- 11 Hermanns S, Reiprich P, Muller HW. A reliable method to reduce collagen scar formation in the lesioned rat spinal cord. *J Neurosci Methods* 2001; **110**: 141–146.
- 12 Lee YS, Lin CY, Robertson RT, Hsiao I, Lin VW. Motor recovery and anatomical evidence of axonal regrowth in spinal cord repaired adult rats. *J Neuropathol Exp Neurol* 2004; **63**: 233–245.
- 13 Teng YD, Lavik EB, Qu X, Park KI, Ourednik J, Zurakowski D *et al*. Functional recovery following traumatic spinal cord injury mediated by a unique polymer scaffold seeded with neural stem cells. *Proc Natl Acad Sci USA* 2002; **99**: 3024–3029.
- 14 Noble LJ, Wrathall JR. Spinal cord contusion in the rat: morphometric analyses of alterations in the spinal cord. *Exp Neurol* 1985; **88**: 135–149.
- 15 Schucht P, Raineteau O, Schwab ME, Fouad K. Anatomical correlates of locomotor recovery following dorsal and ventral lesions of the rat spinal cord. *Exp Neurol* 2002; **176**: 143–153.
- 16 You SW, Chen BY, Liu HL, Lang B, Xia JL, Jiao XY *et al*. Spontaneous recovery of locomotion induced by remaining fibers after spinal cord transection in adult rats. *Restor Neurol Neurosci* 2003; **21**: 39–45.
- 17 Basso DM, Beattie MS, Bresnahan JC. A sensitive and reliable locomotor rating scale for open field testing in rats. *J Neurotrauma* 1995; **12**: 1–21.
- 18 Roy RR, Talmadge RJ, Hodgson JA, Zhong H, Baldwin KM, Edgerton VR. Training effects on soleus of cats spinal cord transected (T12–13) as adults. *Muscle Nerve* 1998; **21**: 63–71.
- 19 Talmadge RJ, Roy RR, Edgerton VR. Persistence of hybrid fibers in rat soleus after spinal cord transection. *Anat Rec* 1999; **255**: 188–201.
- 20 Yu D. A crash course in spinal cord injury. *Postgrad Med* 1998; **104**: 109–110, 113–116, 119–122.
- 21 Kelley MD, Nim S, Rousseau G, Fowles JR, Murphy RL. Early effects of spinal cord transection on skeletal muscle properties. *Appl Physiol Nutr Metab* 2006; **31**: 398–406.
- 22 Canadian Council on Animal Care. *Guide to the Care and Use of Experimental Animals*, 2nd edn, Ottawa, ON, Canada, 1993.
- 23 Thomas AJ, Nockels RP, Pan HQ, Shaffrey CI, Chopp M. Progesterone is neuroprotective after acute experimental spinal cord trauma in rats. *Spine* 1999; **24**: 2134–2138.
- 24 Dalton PD, Shoichet MS. Creating porous tubes by centrifugal forces for soft tissue application. *Biomaterials* 2001; **22**: 2661–2669.
- 25 Dalton PD, Flynn L, Shoichet MS. Manufacture of poly(2-hydroxyethyl methacrylate-co-methyl methacrylate) hydrogel tubes for use as nerve guidance channels. *Biomaterials* 2002; **23**: 3843–3851.
- 26 Smith L. Spectrophotometric assay of cytochrome *c* oxidase. *Methods Biochem Anal* 1955; **2**: 427–434.
- 27 Basso DM, Beattie MS, Bresnahan JC, Anderson DK, Faden AI, Gruner JA *et al*. MASCIS evaluation of open field locomotor scores: effects of experience and teamwork on reliability. Multi-center Animal Spinal Cord Injury Study. *J Neurotrauma* 1996; **13**: 343–359.
- 28 Hase T, Kawaguchi S, Hayashi H, Nishio T, Mizoguchi A, Nakamura T. Spinal cord repair in neonatal rats: a correlation between axonal regeneration and functional recovery. *Eur J Neurosci* 2002; **15**: 969–974.
- 29 Brown KM, Wolfe BB, Wrathall JR. Rapid functional recovery after spinal cord injury in young rats. *J Neurotrauma* 2005; **22**: 559–574.
- 30 Eng LF, Yu AC, Lee YL. Astrocytic response to injury. *Prog Brain Res* 1992; **94**: 353–365.
- 31 Wicks KL, Hood DA. Mitochondrial adaptations in denervated muscle: relationship to muscle performance. *Am J Physiol* 1991; **260**: C841–C850.
- 32 Talmadge RJ, Roy RR, Edgerton VR. Prominence of myosin heavy chain hybrid fibers in soleus muscle of spinal cord transected rats. *J Appl Physiol* 1995; **78**: 1256–1265.