Inquiro, the UAB undergraduate science research journal

Volume 2009 | Number 3 Article 30

2009

Single Walled Carbon Nanotubes as a Regenerative Substrate in Spinal Cord Injury

Jose A. Roman

Robert C. Haddon

Vladimir Parpura

Candace L. Floyd

Single Walled Carbon Nanotubes as a Regenerative Substrate in Spinal Cord Injury

Jose A. Roman[1], Robert C. Haddon[3], Vladimir Parpura[2], Candace L. Floyd[1]
[1]Department of Physical Medicine and Rehabilitation, University of Alabama at Birmingham, Birmingham, USA
[2]Department of Neurobiology, University of Alabama at Birmingham, Birmingham, USA
[3]Department of Chemistry, University of California at Riverside, Riverside, USA

Corresponding author: Candace L. Floyd, Ph.D., Department of Physical Medicine and Rehabilitation, Center for Glial Biology in Medicine, University of Alabama at Birmingham

Abstract:
Traumatic spinal cord injury (SCI) induces tissue damage and results in the formation of a cavity that inhibits axonal regrowth. Filling this cavity with a growth-permissive substrate would likely promote regeneration and repair. Single walled carbon nanotubes grafted with polyethylene glycol (SWNT-PEG) have been shown to increase the length of neuronal processes in vitro and promote growth of neurons in vivo. We hypothesized that an administration SWNT-PEG administered after an SCI contusion injury will promote regeneration of axons into the lesion cavity and functional recovery of the hind limbs. To evaluate this hypothesis, complete transection spinal cord injury was induced at the T9 vertebrae. One week after transection, the epicenter of the lesion was injected with 25µL of either vehicle (saline), 1.0 µg/mL, 10.0 µg/mL, or 100.0 µg/mL of SWNT-PEG. Behavior analysis was conducted before injury, before treatment, and once every seven days for 28 days after treatment. At 28-days post-injection the rats were euthanized and spinal tissue was extracted. Immunohistochemistry was used to detect the area of the cyst, the thickness of the glial scar, and axonal morphology. We found that post-SCI administration of SWNT-PEG increases neurofilament-positive fibers in the lesions and does not increase reactive gliosis. Additionally, post-SCI administration of SWNT-PEG improved hind limb locomotor recovery without inducing allodynia and hyperalgesia. These data suggest that SWNT-PEG may be an effective substrate to promote axonal repair and regeneration after SCI.

Introduction

A hallmark of traumatic spinal cord injury (SCI) is the development of a cystic cavity at the epicenter of the lesion, which is a barrier to subsequent axonal regrowth, regeneration, and functional recovery (Silver et al., 2004). This barrier or reactive gliosis is comprised of astrocytes that serve as both protection against further damage and a blockage against further axonal regrowth and regeneration. We hypothesize that adding a growth permissive substrate to this cavity could promote axonal growth and functional recovery. Single walled carbon nanotubes (SWNTs) are comprised of cylindrical graphene sheets that can range in size from 0.4 nm to 100 nm in diameter and 1 µm to hundreds of µms in length. They can easily be modified to alter biological characteristics such as solubility and polarity depending on their application. Carbon nanotubes (CNTs) have many intrinsic characteristics that make them ideal for engineering capabilities such as small size, high elasticity, high strength, stability, and conductivity. However, CNTs can also be used in a biological context and modified to interact with biological compounds (Malarkey et al., 2007). Previous studies have shown that the attachment of copolymer grafts (functionalization) such as polyethylene glycol (PEG) increases neurite outgrowth and branching *in vitro* (Ni et al., 2005). Although SWNTs have been shown *in vitro* to help stimulate neural regeneration, no *in vivo* experiment has been done to test the regenerative properties of SWNT-PEG in a living organism. Consequently, we evaluated the effect of SWNT-PEG administration on regeneration and repair and functional recovery after SCI in adult rats. The central hypothesis of this study was that administration of SWNT-PEG, either acutely or one week after a spinal cord injury, will promote axonal regeneration/ repair and functional recovery of hind limb locomotion in a rat model.

Materials and Methods

Experimental Groups and Method of Spinal Cord Injury

All surgical protocols were approved by the Institutional Animal Care and Use Committee of the University of Alabama at Birmingham. Fifty-eight adult female Sprague-Dawley rats (250-300g, 2 months old) received a complete transection SCI induced by severing the spinal cord at the T9 vertebral level with a MicroFeather ophthalmic scalpel (Feather Safety Razor Co., Japan). For the acute administration (immediately after SCI) experiment, animal were randomly assigned to the following experimental groups: 1) uninjured control (n=6), 2) complete transection SCI (n=8), 3) complete transection SCI with post-SCI administration of 10 µg/mL of SWNT-PEG in a volume of 25µL (n=5) or 4) complete transection SCI with post-SCI administration of 10 µg/mL of SWNT-PEG in a volume 50 µL (n=5). For the delayed administration experiment, animal received a treatment one week following injury with 25 µL of either vehicle (saline, n=8), 1.0 µg/mL (n=8), 10.0 µg/mL (n=9), or 100.0 µg/mL (n=9) of the SWNT-PEG solution stereotaxically injected into the lesion site epicenter. For all experiments, Matristem tissue sealant (Acell®, Inc. Vet, Columbia, MD) was placed over the transected dura after administration of SWNTs to seal the dura and prevent overflow of SWNT-containing solution into surrounding tissue.

Behavioral Outcome measures

Locomotor and sensory function was assessed weekly after the injection of the SWNTs (post-SCI days 14-35) using the Basso Beattie Bresnahan (BBB) open-field locomotor test, the Noldus Catwalk™ gait analysis, sensitivity to Von Frey filaments and Tail Flick analysis. Hind limb function was assessed with the BBB locomotor test (Basso et al., 1995) pre-injury, and once each week for the following thirty-five days. For this test, animals were placed in a 1.2m diameter metal, smooth-surfaced activity chamber for 4 minutes, and hind-limb movement was scored by two trained investigators who were naïve to the treatment of the animal. All discrepancies in scores were resolved by discussion between the raters at the conclusion of the test and scored to the deficit. Scores were generated for each hind limb and averaged. The BBB test is a gross hind limb locomotor test based on a scale from 0 (no hind limb movement) to 21(normal hind limb movement) that is scored objectively between two trained investigators who are naïve to the treatment. On post-SCI day five prior to administration of SWNT-PEG, in order to standardize the injury among animals, animals with a BBB score greater than 1 were excluded from subsequent analysis. Finer kinematic analysis is computed using The Catwalk™ gait analysis (Hamers et al., 2006). Animals traverse a glass walkway and a high speed camera digitally records the paw prints in real time. The gait is then analyzed, frame by frame, with the assistance of integrated software. One week preceding the injury, the animals were introduced to the procedure and a baseline result was obtained one day pre-injury, and then subsequent analysis was conducted once every week following the injury for thirty-five days. Mechanical allodynia (hypersensitivity to an originally non-noxious stimulus) was measured by determining the animal's sensitivity to von Frey filaments (Chaplan et al., 1994). By applying a stimulus force of 2 g, 4g, 6g, 8g, 15g, 26g, or 60g, the plantar surface of the hind paw was evaluated for a positive response- sensation by lifting the paw. The lowest filament receiving at least 50% positive response of five trials (at least three positive responses) was recorded as the conclusive sensitivity level. Only animals with plantar placement in the BBB test on test day were evaluated Thermal latency was determined by applying radiant heat to the rat's tail. Two tail-flick latencies were obtained per each weekly session. Tail Flick analysis was used to determine the sensitivity to a noxious thermal stimulus when applied to the tail with reaction time and hyperalgesia–an originally noxious stimulus is now more painful (Deakin et al., 1978).

Histological Outcome measures

Spinal cord tissue preparation: At thirty-five days post-SCI, animals were euthanized with an overdose of pentobarbital (Fatal Plus, Vortech Pharmaceuticals, Dearborn, MI) and perfused with 4% paraformaldehyde (PFA). The spinal cord tissue was post-fixed for 24 hours at 4°C in 4% PFA and then cryoprotected in increasing concentrations of sucrose (10-30%) for 48 hours at 4°C. Spinal cords were then frozen over dry ice and then blocked into 5 mm longitudinal sections (2.5 mm rostrally and caudally from the epicenter). Tissue is then embedded in OCT-Compound (Tissue-Tek, Fisher Scientific, Pittsburg, PA, USA) and stored at -80°C. Serial random sections (30µm) were sliced on a cryostat (Global Medical Instrumentation, Inc., Ramsey, MN, USA), and placed on 1%-gelatin coated slides and stored at -20°C until further analysis.

Glial fibrillary acid protein (GFAP) immunohistochemistry: Sections were encircled with a hydrophobic barrier (ImmEdge™, Vector Laboratories, Burlingame, CA, USA) and then dried overnight at room temperature. Slides were rehydrated through graded alcohols, and blocked with an endogenous peroxidase treatment with 0.5% H2O2. Sections were then washed and non-specific reactivity was blocked with 3% goat serum + 0.3% Triton X + 3% bovine serum albumin + 0.1M phosphate buffered saline (PBS) at 37°C for one hour. Tissue sections were rinsed and then incubated in rabbit polyclonal anti-GFAP antibody (1:4000, Z0334, Dako, Carpentaria, CA, USA) for 25 hours at 4°C. Afterwards, the sections were washed in PBS and incubated in Alexa Fluor® 488 goat anti-rabbit secondary antibody (1:4000, A-11008, Invitrogen Co., Carlsbad, CA, USA) at 4°C for 24 hours. Sections were then rinsed, dried overnight, and cover-slipped with Permount mounting media (Fischer Scientific, Pittsburg, PA).

Neurofilament heavy immunohistochemistry: Sections were encircled with a hydrophobic barrier (ImmEdge™, Vector Laboratories, Burlingame, CA, USA) and then dried overnight at room temperature. Slides were rehydrated through graded alcohols, and blocked with an endogenous peroxidase treatment with 0.5% H2O2. Sections were then washed and non-specific reactivity was blocked with 3% goat serum + 0.3% Triton X + 3% bovine serum albumin + 0.1M phosphate buffered saline (PBS) at 37°C for one hour. Tissue sections were rinsed and then incubated in mouse monoclonal to 200 kD Neurofilament Heavy antibody (1:1000, ab24572, Abcam, Cambridge, MA, USA) for 25 hours at 4°C. Afterwards, the sections were washed in PBS and incubated in Alexa Fluor® 488 goat anti-mouse secondary antibody (1:4000, A-11001, Invitrogen Co., Carlsbad, CA, USA) at 4°C for 24 hours. Sections were then rinsed, dried overnight, and cover-slipped with Permount mounting media (Fischer Scientific, Pittsburg, PA).

Stereological quantification

GFAP: Stereological estimates of reactive gliosis rostrally, at the epicenter, and caudally were quantified using relative fluorescence intensity. Relative fluorescence intensity was determined by using a 10x objective on an Olympus BX51 microscope with a motorized stage. The sample size per section was 1mm x 2.5 mm in area. Relative fluorescence intensity per section was computed using StereoInvestigator (MicroBrightField Inc., Colchester, VT). Outlining of the serial sections was conducted by a single investigator naïve to the treatment group.

Neurofilament: Stereological estimates of heavy chain neurofila-

ment positive fibers rostrally and caudally were quantified using the optical fractionator method (West et al., 1991). Neurofilament fiber (NF) counts were performed under a 20x objective on an Olympus BX51 microscope with a motorized stage. NF were counted using StereoInvestigator (MicroBrightField Inc., Colchester, VT). The counting frame was started at a randomized first section and then the inclusion criteria included a NF of minimum length of 5 μm, inclusion in the boundaries set by StereoInvestigator, and in order to minimize error due to irregular slicing NF location within 1 μm of the set z-axis. Outlining of the serial sections was conducted by a single investigator naïve to the treatment group. The total approximation by optical fractionator and the second coefficient of error were calculated as estimates and a value of 0.1 for the second coefficient of error was accepted.

Statistical Analysis

Data are shown as mean ±standard error of the mean and were analyzed using SigmaPlot® (v11, Systat Software Inc., San Jose, CA, USA). Data were analyzed using the independent two-sample student's t-test. Statistical significance was set at $p<0.05$.

Results

Acute administration of SWNT-PEG promotes axonal repair and regeneration without inducing gliosis

In the first experiment, we evaluated acute post-SCI administration of SWNT-PEG on markers of axonal repair. The tissue of animals that received either a laminectomy, complete transection and no treatment with SWNT-PEG, or treatment with 10 μg/mL of SWNT-PEG at 25 μL or 50 μL had immunohistochemistry analysis conducted on serial sections with an antibody against heavy neurofilament (NF). Figure 1 shows a representative micrograph of a longitudinal section of rat spinal cord at the epicenter of the lesion processed with immunohistochemistry for neurofilament (panel A). Stereological quantification of neurofilament positive fibers showed that administration of 25 or 50 μL of 10 μg/mL SWNT-PEG induced a significant increase (indicated by +) in neurofilament positive fibers compared to the non-treated, injured animals (SCI + Sealant). Non-treated animals also showed a significant decrease in NF-positive fibers (indicated by *) compared to uninjured animals (LAM). These data suggest that treatment with SWNT-PEG promotes axonal repair and regrowth into the cavity after a spinal cord injury.

In a second set of serial sections, immunohistochemistry analysis with an antibody against glial fibrillary acidic protein (GFAP) was conducted. Figure 2 shows a representative micrograph depicting a longitudinal section of rat spinal cord at the epicenter of the lesion processed with immunohistochemistry for GFAP (panel A). Quantification of GFAP-reactivity with relative fluorescence intensity showed that treatment with SWNT-PEG induced GFAP relative fluorescence intensity values similar to that of the non-treated and non-injured groups, suggesting that SWNT-PEG administration acutely post-SCI does not induce gliosis.

Delayed administration of SWNT-PEG promotes functional recovery of hind limb locomotion

After determining that acute SWNT-PEG promoted axonal repair and regrowth, we evaluated a more clinically relevant delayed administration with treatment one week post-injury. Treatment of 25 μL with either saline, 1.0 μg/mL, 10.0 μg/mL, or 100.0 μg/mL was administered one week post-injury. Behavior was evaluated once a week for twenty-eight days after SWNT-administration (35 days after SCI). The BBB test was performed weekly for thirty-five days post-injury. As shown in figure 3, animals receiving SWNT-PEG at either 1.0, 10.0, or 100.0 μg/mL had improved BBB scores as compared to animals that received no treatment (0μg/mL). This suggests that delayed post-SCI administration of SWNT-PEG caused increased recovery of locomotor function compared to animals that received no treatment. Figure 4 shows the results of the Catwalk™ kinematic analysis of fore to hind limb coordination as evaluated using the regularity index. The regularity index indicates the percentage of steps that are placed in a regular, coordinated step pattern. Animals that received the 100mg/mL treatment of SWNT-PEG showed a trend toward greater fore to hind limb coordination, but this did not reach statistical significance. Hind paw print area, which indicates the contact area of the plantar surface of the paw in stepping whereby larger print areas indicate more plantar contact, was also analyzed using the Catwalk™ kinematic system. As shown in Figure 5, animals that received the 100 μg/mL treatment showed a trend toward improvement in maximum paw print area at day 35 day post-injury. These data suggest that the 100mg/mL concentration of SWNT-PEG may improve hind-limb kinematics, an indicator of improved hind limb locomotor recovery.

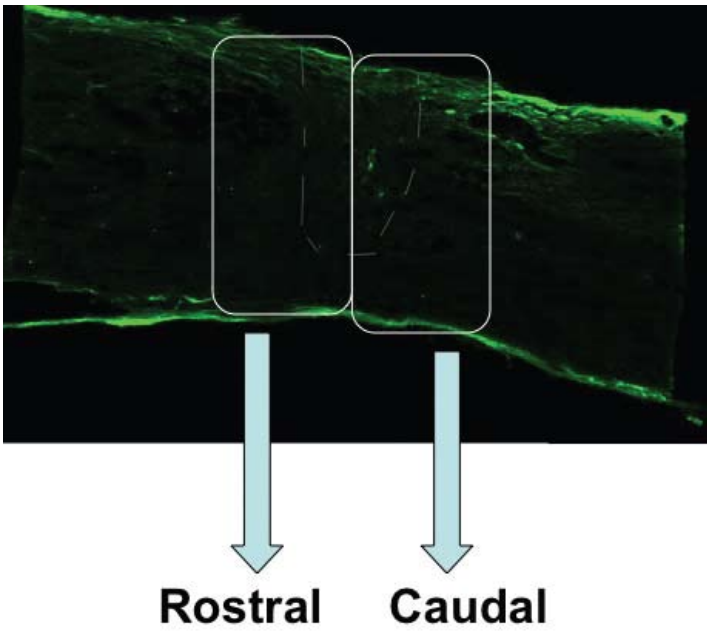
Post-SCI administration of SWNT-PEG does not induce neuropathic pain

Allodynia was evaluated using von Frey filaments. Figure 6 shows the quantification of the sensitivity to a stimulus using the von Frey filaments. No decrease in mechanical threshold over pre-injury assessment was observed suggesting no allodynia was induced. Figure 7 shows the latency of a response due to a thermal stimulus in the tail flick assay. After SCI, reaction time to the thermal stimulus decreased, suggesting that SCI caused hyperalgesia. Administration of SWNTs after SCI did not alter the response to thermal stimulus which suggests no induction of neuropathic pain below the level of the SCI lesion.

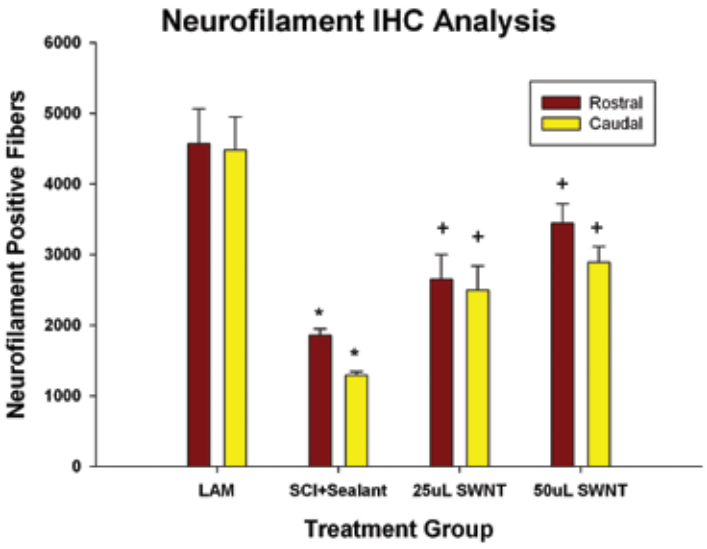
Discussion

In this study, we sought to evaluate a growth permissive substrate that would enhance the regrowth of damaged axons after injury. We first report that acute post-SCI administration of 10.0 μg/mL of SWNT-PEG at both volumes (25 μL and 50 μL) was shown to have statistically more neurofilament growth compared to the injured, non-treated group suggesting that SWNT-PEG increases axonal regrowth. Another factor to determine whether SWNT-PEG could be an effective treatment for spinal cord in-

Figure 1: SWNT-PEG animal showed increase in neurofilament-positive fibers.

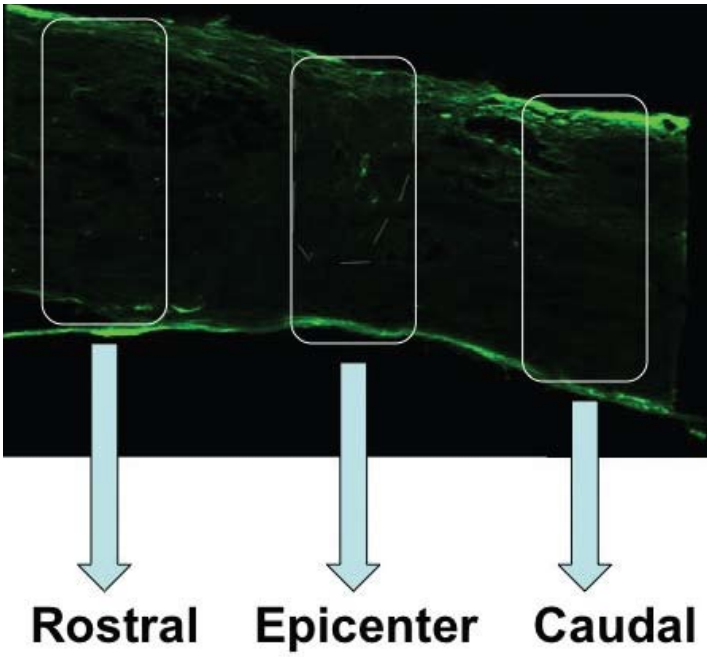


Panel A: Representative transverse section of rat spinal cord at the epicenter of the lesion indicating regions of interest wherein neruofilament positive fibers were quantified. Regions of interest 1mm rostral (red) and caudal (yellow) to the lesion site were quantified using stereological techniques to determine numbers of NF-positive fibers among all four treatment groups of SWNT-PEG.

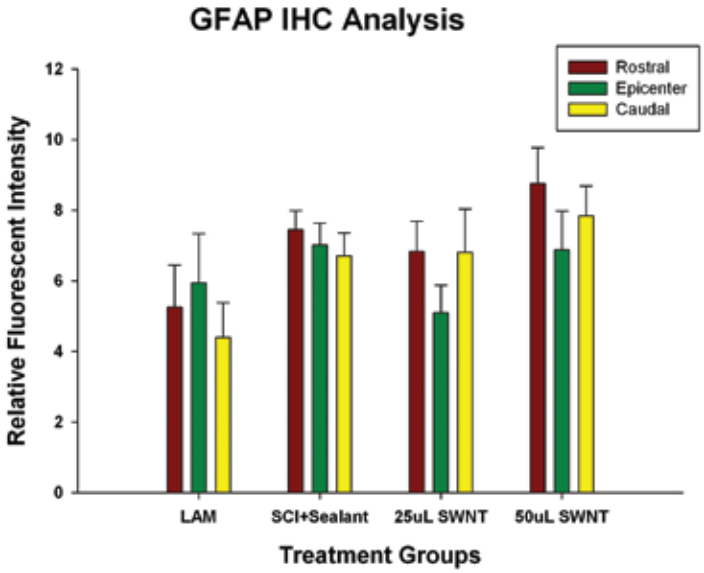


*Panel B: Stereological quantification of NF-positive fibers was conducted using optical fractionator. SCI caused a significant reduction in the number of NF-fibers (SCI + Sealant) as compared to the uninjured animals (LAM), indicated by *. Post-SCI administration of SWNT-PEG significantly increased the numbers of NF-positive fibers, indicated by + (n=5-8 per group).*

Figure 2: SWNT-PEG does not induce reactive gliosis.



Panel A: Representative transverse section of rat spinal cord at the epicenter of the lesion indicating regions of interest wherein GFAP immunoreactivity was quantified. Sections 1mm rostral (red), epicenter (green), and caudal (yellow) to the lesion site were quantified by measuring the relative fluorescent intensity in regions of interest (1mm x 2.5mm) among all four treatment groups of SWNT-PEG.



Panel B: Quantification of GFAP-immunoreactivity indicated that no volume of SWNT-PEG altered relative fluorescence intensity which suggests that treatment with SWNT-PEG did not increase reactive gliosis (n=5-8 per group).

→ top of page 74

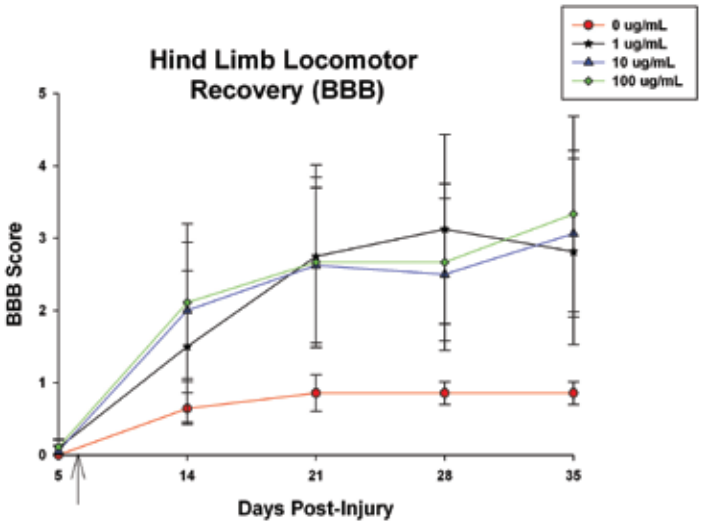


Figure 3: SWNT-PEG treated animals showed improved recovery of hind limb locomotion in the open field.

Hind limb locomotor function was evaluated in the open field and scored using the BBB test. Post-SCI evaluation was conducted once prior to treatment (day 5) and weekly after SWNT-PEG administration (days 14, 21, 28, 35). Arrow indicates time of SWNT-PEG administration (post-SCI day 7). Animals receiving SWNT-PEG were found to regain locomotor function compared to animals that received no treatment (0 ug/mL), suggesting that treatment with SWNT-PEG promotes hind limb recovery (n=8-9 per group).

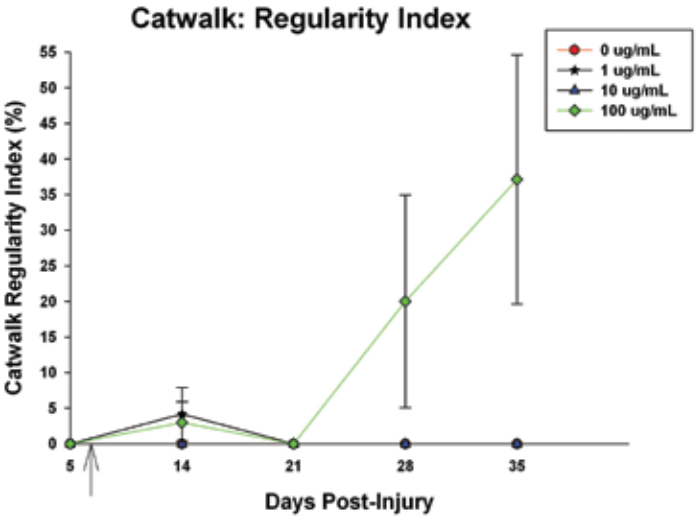


Figure 4: Post-SCI administration with 100 µg/mL of SWNT-PEG improved locomotor coordination in the Catwalk task.

The Catwalk™ gait analysis and integrated software analyzed fine kinematic paw placements and computes various measures such as regularity index. Kinematic analysis of fore to hind limb coordination was evaluated using the regularity index. The regularity index indicates the percentage of steps that are placed in a regular, coordinated step pattern. Animals that received the 100 µg/mL treatment showed a slight improvement in regularity index at 28 and 35 days post-injury, suggesting that treatment with 100 µg/mL of SWNT-PEG slightly improves locomotor coordination (n=8-9 per group).

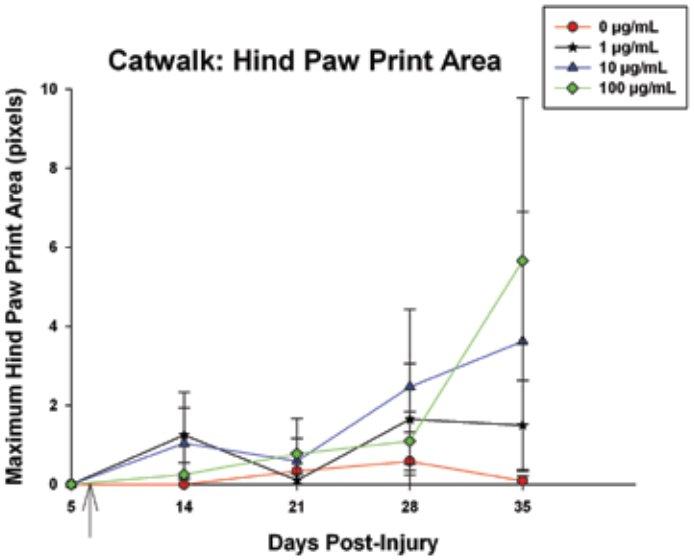


Figure 5: Post-SCI administration of SWNT-PEG produced a trend toward increased plantar placement of the hind paw in the Catwalk task.

The Catwalk™ gait analysis and integrated software analyzed fine kinematic paw placements and computes various measures such as hind paw print area. Kinematic analysis of maximal hind paw print area was conducted prior to SCI (data not shown), after SCI, and post-SCI administration of SWNT-PEG (indicated by arrow). The hind paw print area indicates the contact area of the plantar surface of the paw in stepping and larger print areas indicate more plantar contact. Animals that received the 100 µg/mL treatment showed a trend toward improvement in maximum paw print area at day 35 day post-injury, suggesting that treatment with 100 µg/mL of SWNT-PEG slightly improves plantar placement of the hind paw (n=8-9 per group).

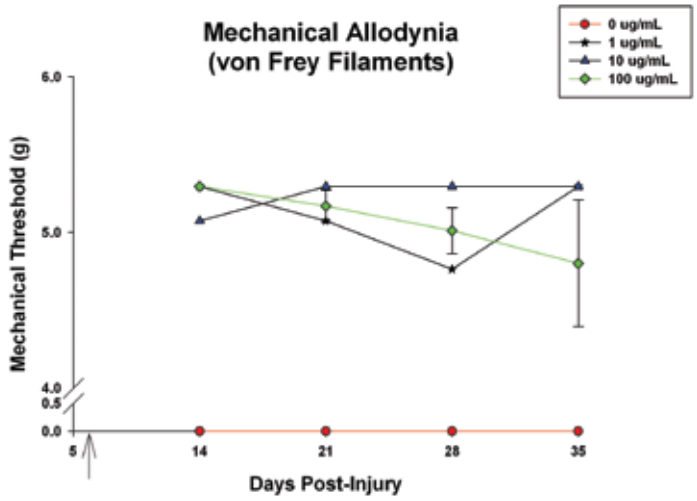


Figure 6: (bottom of previous page) SWNT-PEG treated animals showed recovery of sensation but no allodynia.

A non-noxious light touch of the plantar surface of the paw with von Frey filaments was used to assess hind limb sensation and mechanical allodynia. The withdrawal threshold in response to mechanical stimulation was quantified. Only animals with plantar placement on test day were evaluated. Pre-SCI values are indicated on day 0 and the post-SCI administration time (day 7) of SWNTs is indicated with the arrow. Rats treated with SWNT-PEG responded to a mechanical stimulus similar to the original response prior to injury (day 0) and greater than rats than received vehicle. No decrease in mechanical threshold over pre-injury assessment was observed suggesting no allodynia was induced (n=1-2 per group).

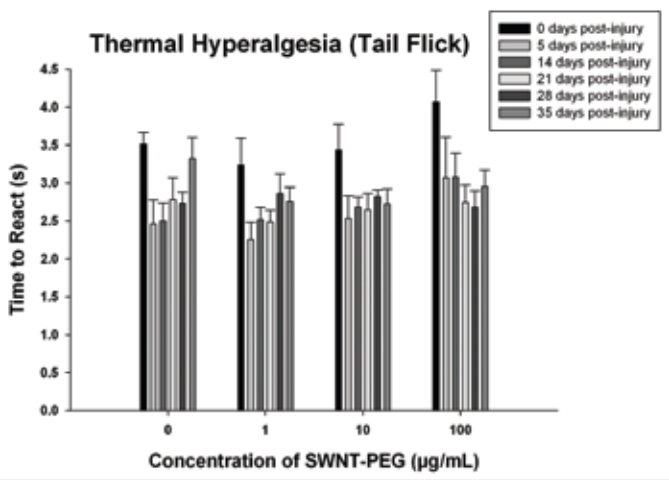


Figure 7: Post-SCI SWNT-PEG administration did not alter reaction to a noxious stimulus, hyperalgesia.

Sensitivity to a noxious thermal stimulus applied to the tail was assessed with reaction time. The latency to move the tail out of the heated light was measured before (day 0) and after SCI (days 14-35) and then weekly after administration of SWNT-PEG (post-SCI days 7, 14, 21, 28 and 35). After SCI, reaction time to the thermal stimulus decreased, suggesting that SCI caused hyperalgesia. Administration of SWNTs after SCI did not alter the response to thermal stimulus (n=8-9 per group).

jury is a change in reactive gliosis levels since this is a key element in scar formation and can limit the permissiveness of the graft (Nieto-Sampedro, 1999). We report that acute post-SCI administration of SWNT-PEG did not induce reactive gliosis.

After determining that administration with 10 µg/mL of SWNT-PEG was effective in increasing neurofilament positive fibers and did not cause an increase reactive gliosis, a more clinically relevant administration time point of one week was evaluated. Weekly behavioral analysis was conducted using the BBB test, Catwalk™ kinematic analysis, sensation with the von Frey filaments, and Tail-Flick assay. We found that very little functional recovery of hind limb locomotion occurred in animals treated with saline; however, all three SWNT-PEG treatment groups showed slightly better improvement. Although the increase in BBB score from a score less than 1 (control group) to a score of 3 (SWNT-PEG treated groups) is modest, this represents functional recovery from no observable movement in the hind limb to extensive movement of two joints of the hind limb, most often the hip and knee. These data suggest that SWNT-PEG improves hind limb locomotor recovery post-spinal cord injury, albeit modestly. In further kinematic analysis using the Catwalk™ gait analysis, we found that a treatment with 100 µg/mL SWNT-PEG induced a trend toward an increase in regularity index and hind paw print area in days twenty-eight and thirty-five days post-injury. This suggests that a treatment with 100 µg/mL of SWNT-PEG may improve hind limb locomotor recovery and may be the best dose for further exploration. In addition, because SWNT-PEG had not been tested *in vivo* after SCI, pain needed to be assessed before and after injury and treatment with SWNT-PEG. We evaluated the mechanical threshold using von Frey filaments before and after injury and treatment and there was no significant difference suggesting that treatment with SWNT-PEG does not induce mechanical allodynia. An important limitation of these data evaluating sensory response is that very few animals were included in the analysis as the test requires the ability to plantar place the hind paw. Since most animals did not regain a level of hind limb function that was sufficient for plantar placement, only a fraction of the animals used for other tests were evaluated. We also assessed the latency to move the tail after application of thermal stimulus before and after injury and treatment as well. We report that all four groups show the same trend of a decrease in latency immediately after injury and a gradual increase in latency following treatment. This data suggest that treatment with SWNT-PEG does not induce hyperalgesia.

In conclusion when taken together, these data indicate that SWNT-PEG, especially at a concentration of 100 µg/mL, improves axonal repair and regeneration into the lesion cavity and induces modest functional recovery, which suggests that this may be an effective substrate to promote axonal repair and regeneration after SCI. Future studies will include: analysis of tissue sparing and regeneration using immunohistochemistry on animals treated one week post-injury and evaluation of the administration of SWNT-PEG into a chronic SCI in a rat model. Additionally, the addition of growth factors and/or growth supporting cells may improve the extent of axonal repair and regeneration as well as functional recovery which makes it possible that SWNT-PEG may be an excellent candidate for a combinational therapy approach to repairing the injured spinal cord.

Acknowledgements

This study was supported by the UAB BioMatrix Engineering and Regenerative Medicine (BERM) Center Pilot Grant, the Center for Glial Biology in Medicine, and the Department of Physical Medicine and Rehabilitation. This study was also partially supported by the Ronald E. McNair Post-Baccalaureate

Achievement Program and the Wachovia Scholars Foundation Fund. Special thanks to Tracy Niedzielko for assistance with behavioral analysis.

Reference List

Basso, D. M., M. S. Beattie, and J. C. Bresnahan (1995). "A sensitive and reliable locomotor rating scale for open field testing in rats." J. Neurotrauma 12: 1-21.

Chaplan, S. R., et al (1994). "Quantitative assessment of tactile allodynia in the rat paw." J. Neurosci. Methods 53: 55-63.

Deakin, J. F. and J. O. Dostrovsky (1978). "Involvement of the periaqueductal grey matter and spinal 5-hydroxytryptaminergic pathways in morphine analgesia: effects of lesions and 5-hydroxytryptamine depletion." Br. J. Pharmacol. 63: 159-165.

Hamers, F. P., G. C. Koopmans, and E. A. Joosten (2006). "CatWalk-assisted gait analysis in the assessment of spinal cord injury." J. Neurotrauma 23: 537-548.

Malarkey, E. B. and V. Parpura (2007). "Applications of carbon nanotubes in neurobiology." Neurodegener. Dis. 4: 292-299.

Ni, Y., et al (2005). "Chemically functionalized water soluble single-walled carbon nanotubes modulate neurite outgrowth." J. Nanosci. Nanotech. 5: 1707-1712.

Nieto-Sampedro, M (1999). "Neurite outgrowth inhibitors in gliotic tissue." Adv. Exp. Med. Biol. 468: 207-224.

Silver, J. and J. H. Miller (2004). "Regeneration beyond the glial scar." Nat. Rev. Neurosci. 5: 146-156.

West, M. J., L. Slomianka, and H. J. Gundersen (1991). "Unbiased stereological estimation of the total number of neurons in thesubdivisions of the rat hippocampus using the optical fractionator." Anat. Rec. 231: 482-497.