34th Annual International Conference of the IEEE EMBS
San Diego, California USA, 28 August - 1 September, 2012

DTI for assessing axonal integrity after contusive spinal cord injury and transplantation of oligodendrocyte progenitor cells

Faith A. Bazley, *Student Member*, *IEEE*, Amir Pourmorteza, *Student Member, IEEE*, Siddharth Gupta, Nikta Pashai, Candace Kerr, and Angelo H. All, *Member*

Abstract— **We describe the feasibility of using diffusion tensor magnetic resonance imaging (DT-MRI) to study a contusive model of rat spinal cord injury following human stem cell transplantation at and around the site of injury. Rats receiving either a laminectomy or contusion injury were transplanted with oligodendrocyte precursor cells (OPCs). During the course of the study, bioluminescence imaging (BLI; up to 100 days) and somatosensory evoked potentials (SSEPs; up to 42 days) were used to evaluate cell survival and functional outcomes. Spinal cords were then analyzed *ex vivo* upon termination using diffusion tensor imaging (DTI). Improvements in fractional anisotropy (FA) at day 100 post-transplantation corresponded with cell survival and functional SSEP improvements. Thus, we illustrate the feasibility of DTI for evaluating axonal integrity in SCI after cell replacement therapies, and we provide examples utilizing OPC transplantations in a contusion rat model.**

I. INTRODUCTION

More than two-thirds of all human spinal cord injuries (SCI) are contusive. Rat models of contusive SCI represent a clinically relevant model for studying human SCI, as they closely mimic the pathology and progression of injury in humans [1]. The amount of spared and functional white matter tissue, which is composed of ascending and descending axonal tracts, that remains after SCI correlates well with the extent of recovery post-trauma [2]. Unlike transection models for which every axon is severed, in contusions, a number of axons survive primary injury and are anatomically continuous through the injury epicenter but demyelinated and therefore not functional. In the weeks following initial trauma, secondary injury mechanisms including inflammation, macrophage infiltration, and glial scaring continue to damage axons and destroy their surrounding conductive myelin that is essential for signal transduction. Therapeutic strategies aimed at preventing or limiting the secondary injuries and remyelination may be able to restore function to spared fibers that span the lesion following a contusive SCI [3]. One promising strategy is utilizing stem cells to derive OPCs for replacement into the SC with the aim of remyelinating native axons that have lost function due to oligodendrocyte apoptosis and demyelination *in vivo*. However, reaching this aim will require a thorough understanding axonal fiber sparing, secondary injury, and transplanted cell dynamics. Thus, it is important to characterize the epicenter of injury and to determine the optimal strategies and logistics for feasible cell transplantation therapies.

DT-MRI has allowed for major advancements for studying specific pathways of the CNS. The technique is based on the diffusion principles of water and enables researchers to investigate and map specific pathways and microstructures of white matter by measuring water diffusivity along defined regions of interest (ROIs). Normal water diffusion is isotropic such that molecules diffuse non-preferentially in all directions of 3D space. However, structures such as axonal fiber tracts are able to guide the diffusion of water molecules such that they preferentially move in specific directions parallel to the tracts, or anisotropically. New acquisition techniques such as echo-planar imaging allow for reductions in motion artifacts, improved spatial resolution, and the construction of 3D neural pathways through the spinal cord [4] for quantifying the amount of spared pathways following SCI [5-8].

In this study, we utilized diffusion tensor imaging (DTI) to visualize the spinal cord white matter axonal fibers *ex vivo* up to 100 days after induction of a moderate contusion SCI in rats. We illustrate how DTI provides significant benefits over traditional T2-weight MRI and show how DTI can be used to evaluate the extent of axonal damage and subsequent repair, based on fractional anisotropy (FA). Finally, we show that DTI is a powerful tool for evaluating OPC transplantation therapy for SCI. We used bioluminescence cell tracking to verify cell survivability in conjunction with electrophysiology to show potential functional improvements, both of which corroborate our DTI data. Our data provides preliminary work for further studies of OPC therapies, and demonstrates how these techniques can be used to evaluate the long-term effects and benefits such therapies in a rat model of contusive SCI.

Manuscript received March 29, 2012. This work was supported by the Maryland Stem Cell Research Fund under Grants 2007-MSCRFII-0159-00 and 2009-MSCRFII-0091-00.

F. A. Bazley, A. Pormourteza, S. Gupta, N. Pashai, and A. H. All (phone: 410-502-5393; e-mail: angelo@bme.jhu.edu) are with the Dept. of Biomedical Engineering, Johns Hopkins University, Baltimore, MD, USA A. H. All is also with the Dept. of Neurology, Johns Hopkins University School of Medicine, Baltimore, MD, USA. C. Kerr is with the Dept. of Biochemistry & Molecular Biology, University of Maryland, Baltimore, MD, USA.

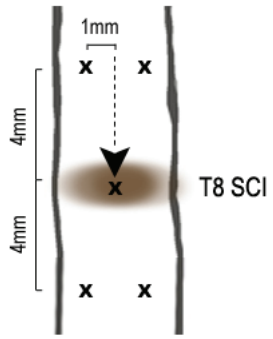

Fig. 1 Schematic illustration of the contusion spinal cord injury induced at the thoracic 8 level. Arrowhead: location of impact inducing the SCI. X: locations of OPC transplantations following injury.

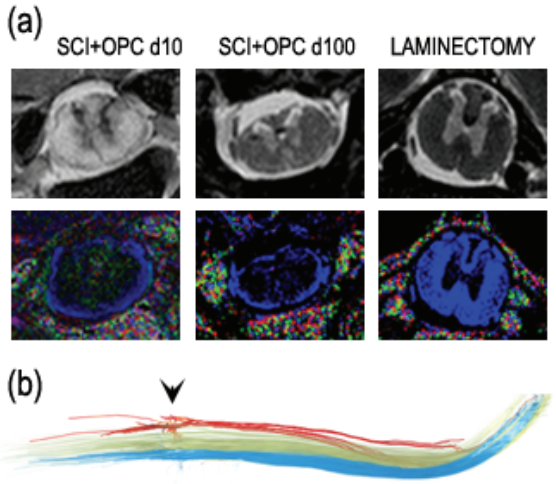

Fig. 2 (a) Comparison of traditional T2-weighted MRI (top) and the color map generated by diffusion tensor MRI (bottom), all taken through the epicenter of injury or the site of laminectomy. Blue in the DTI images indicates water diffusion in the axial direction (perpendicular to the image plane). (b) A representative DT-MR image of fiber tracking showing the dorsal (red), lateral (green), and ventral (blue) pathways in an injured rat that received OPC transplantation after the contusion. The image was taken at day 10 after transplantation and illustrates the extensive axonal damage to dorsal pathways with less damage to lateral and ventral pathways.

II. Methods

A. Laminectomy and spinal cord injury

Rats were anesthetized with 1.5% isoflurane and underwent laminectomy. An incision was made in the skin and paravertebral muscles at T7-9, the lamina of T8 vertebrae was carefully removed such that the dura remained intact. Next, rats were randomly divided into two groups: those receiving a moderate contusion injury (12.5 mm injury via a MASCIS-impactor weight-drop device; n=8) and sham controls receiving only laminectomy (n=5).

B. Oligodendrocyte progenitor transplants

Constitutive undifferentiated human embryonic stem (hES) cells (2.5×10^6 H1 cells from Wicell, Madison WI) were cultured on 75 cm^2 polystyrene tissue culture dishes (Corning, NY) at 37°C and 5% CO_2, then expanded in ES-cell growth media and differentiated into OPCs, as we have previously described [9, 10]. Rats were immunosuppressed with daily s.c. injections of 14 mg/kg cyclosporine, and approximately 125,000 cells/µl were transplanted 2 hrs after injury using a 10-µl syringe (7635-01, Hamilton, Reno, NV). The cells were injected at a controlled rate of 1 µL/min at five injection sites (**Fig. 1**) 1.5 mm below the surface of the cord. The paravertebral muscles and skin were then sutured and closed in layers, and the rat was given appropriate post-operative treatment.

C. MRI and fiber tracking

For *ex vivo* assessment of SCI, rats were sacrificed, spinal cords extracted, and T2-weighted MRI and DTI were performed at days 10 and 100 post-injury using a vertical 9.4 Tesla NMR spectrometer. Diffusion tensor data were acquired using a multi-slice diffusion-weighted spin echo sequence with parameters TE = 27 ms, TR = 3000 ms, bandwidth = 100 kHz, matrix size = 160x160, slice thickness = 0.5 mm, 8 signal averages. First, the diffusion-weighted images were registered to the T2-weighted image using the automatic image registration algorithm to correct for distortions. ROIs were manually selected encompassing dorsal, ventral, and lateral white matter areas, and FA was calculated on a voxel-by-voxel basis from the diffusion tensor using DTI Studio (JHU, Baltimore, MD) [11] software. A volume of interest was created by combining sequential 2D masks defined manually by the outer edge of the SC in axial T2-weighted images.

D. Bioluminescence imaging

A subset of hES cells were transfected for 6 hours with the lentiviral vector, pLenti4-CMV-fLuc2, in the presence of 0.1% polybrene (Chemicon, MA) to induce the expression of firefly *luciferase* and to allow for cell tracking.

Three injured and 4 laminectomy rats received OPC-*luciferase* transplants and underwent BLI using a Xenogen IVIS-200 Optical In Vivo Imaging System (Caliper Life Sciences, Hopkinton, MA). Rats were anesthetized and administered an i.p. injection 300 mg/kg D-luciferin, and a small skin incision was made to expose the site of SCI. A series of images capturing luciferin light intensity were acquired, and the peak intensities were quantified as total flux (photons/second) using Living Image 3D Analysis (Xenogen Corporation, Caliper Life Sciences) software.

E. SSEP recording

Somatosensory evoked potentials were recorded from the right hindlimb region of the sensorimotor cortex upon stimulation of the right tibial nerve in (injured) hindlimbs. Prior to laminectomy, transcranial screw electrodes were implanted in the cranium and mounted on an electrode pedestal with dental cement, as previously described [12-17]. Two baseline SSEPs were recorded prior to injury, then on days 1, 14, and 42 after injury to monitor longitudinal changes in sensory function. Intramuscular needle electrodes were inserted near the tibial nerve of the hindlimb, which were stimulated using an isolated constant current stimulator (3.5 mA, 200 µs duration, 0.5 Hz; Digitimer, Hertfordshire, UK). SSEPs were recorded at 4882 Hz using a commercially available system (Tucker Davis, Alachua, FL) and processed using MATLAB, as previously described [12, 13].

III. Results

Following SCI and cell transplantation, rats underwent MRI and DTI. Fiber tracts are clearly visible in the acquired DTIs, whereas T2-weighted MRI fails to resolve spared axonal tracts in the white matter especially in the presence of hematoma and inflammation in the first weeks after injury (**Fig. 2(a)**). In addition, the FA values derived from the DTI axial slices of the spinal cord can be used to reconstruct a 3D view such that the spared and damaged fibers can be easily visualized. **Fig. 2(b)** illustrates that the majority of the damage occurred to the gray matter at the epicenter of injury and to the immediate vicinity of dorsal pathways, whereas lateral and ventral pathways remained more preserved following contusion.

A subset of rats were randomly chosen to receive OPC transplants to evaluate the feasibility of utilizing DTI for assessing beneficial outcomes attributed to cell therapies. Ten days after injury, both the treated rat and untreated rat exhibited a large decrease in FA at the epicenter of injury, clearly illustrated by the FA peak minima for the dorsal, ventral, and lateral columns shown in **Fig. 3(a)**. However, 100 days after injury and OPC cell-replacement treatment, the magnitude of this peak is reduced, which signifies increased anisotropic movement of water along repaired axonal pathways. Yet regions caudal and rostral to the epicenter exhibited a decrease in FA, which suggests the spread of damage that is known to occur in the weeks to months post-injury. Thus, the improvement observed at the epicenter is accompanied by a slight loss of axonal integrity caudal and rostral to the epicenter by day 100 post-injury and transplant.

Bioluminescent imaging of rats receiving *luciferase*-transfected OPCs verified cell survivability and proliferation following transplantation up to 100 days post-injury (**Fig. 3(b)**). Following an initial drop in cell density (3.12×10^6 photons/s to 3.66×10^4 photons/s), likely due to cellular death caused by the hostile microenvironment of the SCI, the OPCs not only survive but proliferate and reach a final luminescence of 9.12×10^7 photons/s by day 100.

Fig. 4 shows somatosensory evoked potentials (SSEPs) that were longitudinally recorded from two rats, one receiving OPC treatment and the other receiving no treatment post-injury. These preliminary results show an improvement in conduction, marked by the increased SSEP amplitude at 42 days, for the OPC-transplantation rat compared with the control receiving no-treatment.

IV. Discussion

Although traditional MRI has often been considered the modality of choice for visualizing spinal cord tissue damage, it is not sensitive enough to evaluate axonal integrity following SCI. However, subtle geometric changes within underlying neural tissue structure and pathologies such as traumatic brain injury [18] and neurological diseases [19] can be measured using DTI. A thorough investigation of diffusion of the entire spinal cord from the acute through the chronic stages of SCI has been reported [7] but feasibility for use in evaluating cell transplantation therapies remains to be determined.

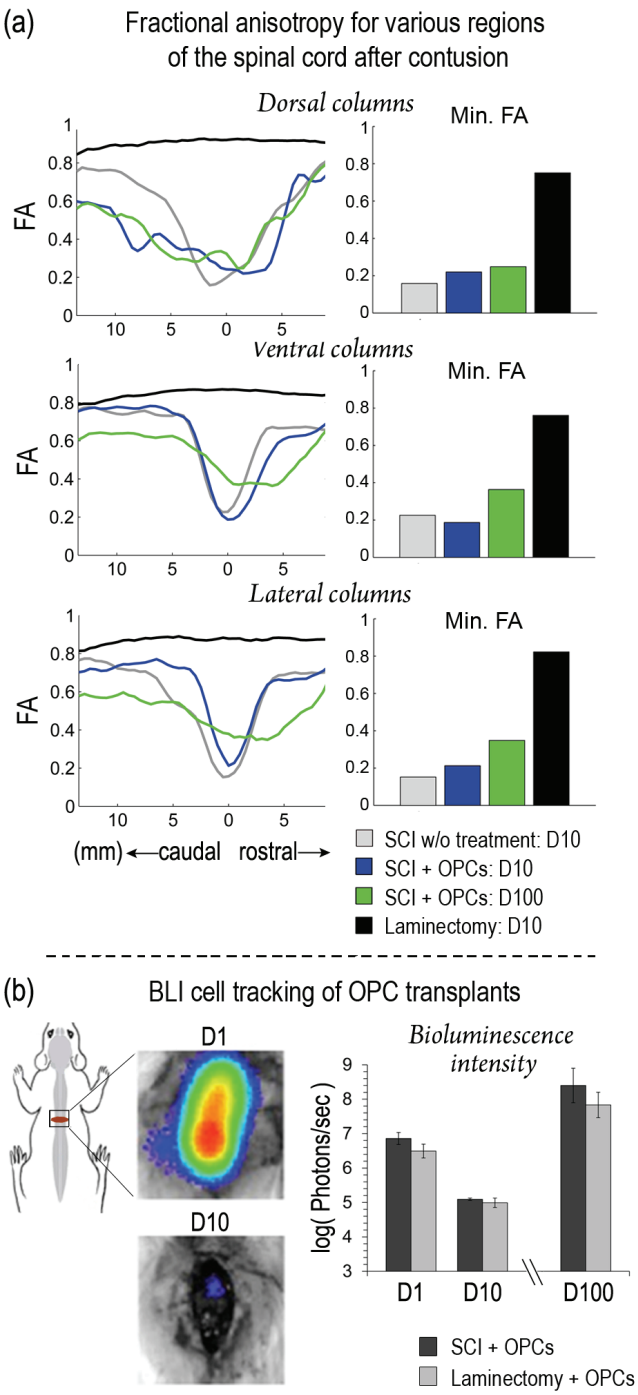

Fig. 3 (a) FA of 3 animals (n=1 for each group) for *ex vivo* spinal cord segments. Left: FA values across the entire extracted segment; peak minimums at x=0 correspond to the epicenter of injury and are clearly visible at day 10. FA of laminectomy remains near 1 indicating that axonal pathways were not injured. Right: bar plots of FA peak minimums; FA is increased in the lateral and ventral columns after 100 days following OPC transplants compared with no treatment at day 10, which may be due to remyelination of axonal pathways after SCI. (b) BLI verified survival of transplanted *luciferase*-transfected OPCs up to 100 days. Left: schematic showing location of T8 injury and representative bioluminescence images for one rat; right: quantified results for OPC-treated (n=3) and laminectomy controls (n=4).

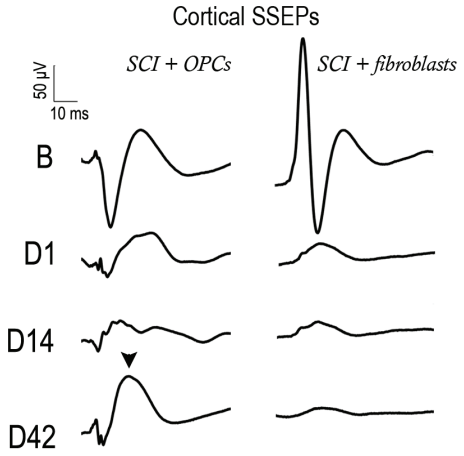

Fig. 4 Cortical SSEPs measured from right hindlimb regions of the sensorimotor cortex upon stimulation of the right hindlimb. SSEPs were longitudinally measured for 42 days for 2 rats receiving moderate contusive SCI, 1 with an OPC transplant. Arrowhead: improved amplitude may be due to remyelination of spared axons, leading to better sensory conduction.

Here, FA was shown to be a good measure for evaluating axonal integrity, and we were able to detect a significant increase in FA from day 10 to day 100 post-SCI. At day 10 after SCI and OPC transplantation, there was little observable difference in FA between OPC-treated and no-treatment rats. This is expected, as any functional benefit of remyelination will likely occur several weeks after transplantation. In contrast, by day 100, the OPC-treated rat showed a marked increase in FA at the epicenter of injury for lateral and ventral regions (0.22 to 0.35 and 0.19 to 0.34, respectively).

To verify the survival of the transplanted OPCs up to 100 days, BLI of OPCs transfected with the luciferase gene was performed for treated rats. BLI revealed a large fraction of the cells that are initially transplanted do not survive the first 10 days, likely due to the hostile microenvironment and elevated inflammation at the site of injury. Cells must be provided sufficient time to integrate, proliferate, differentiate, and reach steady state before they are able to produce myelin that can enwrap existing nude axons post-injury. The increased luminescence at day 100 shows that the OPCs are survived by this time point and may be attributed to the improved FA observed via DTI.

Our supplementary SSEP analysis showed that this potential remyelination may occur by day 42 because by this time point, increased SSEP amplitude was observed for the OPC-transplant rat compared with control.

DTI is technique that is becoming more widespread as neuroscientists seek to better elucidate anatomical changes to neuropathways associated with disease and repair mechanisms. It is widely used to visualize SCI *ex vivo* in rodent models. Here, we illustrate the feasibility of DTI to evaluate potential therapeutic effects of cell replacement therapies, and we offer BLI and SSEP data corroborate our conclusions drawn from DTI results.

REFERENCES

[1] R. Talac, J. Friedman, M. Moore, L. Lu, E. Jabbari, A. Windebank, B. Currier, and M. Yaszemski, "Animal models of spinal cord injury for evaluation of tissue engineering treatment strategies," *Biomaterials,* vol. 25, pp. 1505-1510, 2004.

[2] P. Schucht, O. Raineteau, M. Schwab, and K. Fouad, "Anatomical correlates of locomotor recovery following dorsal and ventral lesions of the rat spinal cord," *Experimental neurology,* vol. 176, pp. 143-153, 2002.

[3] D. J. Donnelly and P. G. Popovich, "Inflammation and its role in neuroprotection, axonal regeneration and functional recovery after spinal cord injury," *Experimental neurology,* vol. 209, pp. 378-388, 2008.

[4] H. Lundell, et al., "Fast diffusion tensor imaging and tractography of the whole cervical spinal cord using point spread function corrected echo planar imaging," *Magnetic Resonance in Medicine,* 2012.

[5] J. Zhang, et al., "Diffusion tensor magnetic resonance imaging of Wallerian degeneration in rat spinal cord after dorsal root axotomy," *The Journal of Neuroscience,* vol. 29, pp. 3160-3171, 2009.

[6] B. M. Ellingson, B. D. Schmit, J. L. Ulmer, and S. N. Kurpad, "Diffusion tensor magnetic resonance imaging in spinal cord injury," *Concepts in Magnetic Resonance Part A,* vol. 32, pp. 219-237, 2008.

[7] B. M. Ellingson, B. D. Schmit, and S. N. Kurpad, "Lesion growth and degeneration patterns measured using diffusion tensor 9.4-T magnetic resonance imaging in rat spinal cord injury," *Journal of Neurosurgery: Spine,* vol. 13, pp. 181-192, 2010.

[8] M. M. Cheung, D. Li, E. S. Hui, S. Fan, A. Y. Ding, Y. Hu, and E. X. Wu, "In vivo diffusion tensor imaging of chronic spinal cord compression in rat model," 2009, pp. 2715-2718.

[9] C. L. Kerr, B. S. Letzen, C. M. Hill, G. Agrawal, N. V. Thakor, J. L. Sterneckert, J. D. Gearhart, and A. H. All, "Efficient differentiation of human embryonic stem cells into oligodendrocyte progenitors for application in a rat contusion model of spinal cord injury," *International Journal of Neuroscience,* vol. 120, pp. 305-313, 2010.

[10] B. S. Letzen, C. Liu, N. V. Thakor, J. D. Gearhart, A. H. All, and C. L. Kerr, "MicroRNA expression profiling of oligodendrocyte differentiation from human embryonic stem cells," *PLoS One,* vol. 5, p. e10480, 2010.

[11] H. Jiang, P. van Zijl, J. Kim, G. D. Pearlson, and S. Mori, "DtiStudio: resource program for diffusion tensor computation and fiber bundle tracking," *computer methods and programs in biomedicine,* vol. 81, pp. 106-116, 2006.

[12] F. A. Bazley, C. Hu, A. Maybhate, A. Pourmorteza, N. Pashai, N. V. Thakor, C. L. Kerr, and A. H. All, "Electrophysiological evaluation of sensory and motor pathways after incomplete unilateral spinal cord contusion," *Journal of Neurosurgery: Spine,* pp. 1-10, 2012.

[13] F. A. Bazley, A. H. All, N. V. Thakor, C. Kerr, and A. Maybhate, "Plasticity Associated Changes in Cortical Somatosensory Evoked Potentials following Spinal Cord Injury in Rats," presented at the 33rd Annual International Conference of the IEEE EMBS, Boston, Massachusetts, 2011.

[14] A. Maybhate, C. Hu, F. A. Bazley, Q. Yu, N. V. Thakor, C. L. Kerr, and A. H. All, "Potential long-term benefits of acute hypothermia after spinal cord injury: Assessments with somatosensory-evoked potentials," *Critical Care Medicine,* vol. 39, 2011.

[15] G. Agrawal, C. Kerr, N. V. Thakor, and A. H. All, "Characterization of graded multicenter animal spinal cord injury study contusion spinal cord injury using somatosensory-evoked potentials," *Spine,* vol. 35, p. 1122, 2010.

[16] G. Agrawal, D. Sherman, A. Maybhate, M. Gorelik, D. A. Kerr, N. V. Thakor, and A. H. All, "Slope analysis of somatosensory evoked potentials in spinal cord injury for detecting contusion injury and focal demyelination," *Journal of Clinical Neuroscience,* vol. 17, pp. 1159-1164, 2010.

[17] A. H. All, G. Agrawal, P. Walczak, A. Maybhate, J. W. M. Bulte, and D. A. Kerr, "Evoked potential and behavioral outcomes for experimental autoimmune encephalomyelitis in Lewis rats," *Neurological sciences,* pp. 1-7, 2010.

[18] M. F. Kraus, et al., "White matter integrity and cognition in chronic traumatic brain injury: a diffusion tensor imaging study," *Brain,* vol. 130, pp. 2508-2519, 2007.

[19] S. E. Rose, et al., "Loss of connectivity in Alzheimer's disease: an evaluation of white matter tract integrity with colour coded MR diffusion tensor imaging," *Journal of Neurology, Neurosurgery & Psychiatry,* vol. 69, pp. 528-530, 2000.