

Combining biological and electrical stimulation therapies following spinal cord injury

Peggy Assinck, BSc;¹ Basem I. Awad, MD;² Roberto Fiorelli, MSc;³ Bradley Lang, BSc⁴

¹Department of Neuroscience, University of British Columbia, Vancouver, Canada; ²Department of Neurological Surgery, Mansoura University Hospital, Mansoura, Egypt; ³Brain Research Institute, University of Zurich/ETH, Zurich, Switzerland; ⁴Department of Neuroscience, Case Western Reserve University, Cleveland, OH

Abstract—The following was completed as part of the 2011 Route 28 Summit at the International Symposium on Neural Regeneration. The topic of the Route 28 Summit was “Novel Ways to Exploit Stem Cells for Recovery of Human Central Nervous System Function.” In response to the Route 28 challenge, we propose a novel combinatorial treatment approach using multiple biological interventions in conjunction with controlled electrical stimulation to enhance the benefits of a cellular replacement strategy. Using an aligned polymer scaffold seeded with embryonic neural stem cells, we aim to create a relay for the disconnected axons in a transection rodent model of spinal cord injury. This approach will be implemented with (1) a growth factor gradient, (2) chondroitinase ABC (chABC) injections, and (3) functional electrical stimulation and in situ-recording. We hope to create an environment that is supportive for stem cell survival and differentiation to facilitate neural relays, long distance host axonal regeneration, and functional recovery.

BACKGROUND

As participants in the Route 28 Summit at the 2011 International Symposium for Neural Regeneration we were asked to use stem cells in a novel way to enhance regeneration. In the days-to-weeks that follow the initial spinal cord injury (SCI), a secondary cascade of deleterious events is initiated, including: local vascular remodeling, electrolyte changes, neurotransmitter accumulation, free radical generation, excitotoxicity, cell death, and loss of neurotrophic factor resulting in substantial damage to the injured region [1–2]. The presence of inhibitory proteins in the environment [3] and the lack of trophic support [4–6] are thought to be some of the factors involved in the lack of central nervous system (CNS) regeneration. In the pre-clinical setting, recent studies have suggested treatments involving

the use of growth factor gradients to guide axons in appropriate directions [7–10], enzymatic digestion of inhibitory chondroitin sulfate proteoglycans (CSPGs) to facilitate axon growth [5] and the use of biodegradable printed scaffolds to enhance survival of transplanted stem cells [11]. However, even when improvements are observed, regeneration is slow, inefficient and never fully restores the nervous system to its pre-injury state in primate models [12–13]. As robust functional recovery is rarely achieved, it is necessary to re-think the stem cell-based SCI regenerative interventions using novel, combinatorial approaches [14].

To close the gap between the alpha motor neurons in the cortex and effector muscles, following SCI, researchers have used functional electrical stimulation (FES), in which electrical stimulation is used to generate or suppress activity within the CNS [15]. FES devices are currently being used in humans to restore: bladder function, upper limb movements including grasping of the hand, posture, balance, cough, and other motor functions [16]. Electrical stimulation near the site of injury has also been shown to facilitate axonal outgrowth [17]. For example, direct stimulation to specific tracts above the level of SCI in a rodent model resulted in robust outgrowth of tract axons and facilitated improved functional recovery [18]. However, the best technologies only restore partial function, which in some cases cause unnatural incomplete movements [19]. In addition, computational and recording technology is yet unable to completely mimic and restore fluid movement after SCI.

To increase the benefits of both biological and FES therapies following SCI, we propose using both in combination. Transplantation of neural stem cells of different origins has been used for several years in SCI [10,20–21] resulting in inconsistent levels of functional restoration. Applying electrical current to the spinal cord can help reactivate circuitry below the injury level and facilitate the control of smooth and skeletal muscle caudal to the injury [22–24]. The premise is that neither therapy by itself yields robust and concerted restoration of function, but the combined strengths of these approaches will achieve this goal.

STUDY PROPOSAL

We hypothesize that combining biological and neuroprosthetic approaches will increase long

distance regeneration of host motor axons, establish a functional relay via exogenous hES-NSCs, and facilitate locomotor recovery following a spinal cord transection in rats.

Specific Aim 1: Creating Sustainable Biological Relays

We first aim to transplant aligned poly-L-lactic Acid (PLLA) matrices seeded with neurally pre-differentiated human embryonic stem cells (i.e., hES-NSCs) in addition to growth factor gradients and chABC injections to create biological relays following spinal cord injury. hES-NSC can be manipulated to direct their fate toward neuronal fates [25–26]. By seeding the scaffold with hES-NSCs, we hope to promote the sprouting and formation of synaptic connections with intact circuits on either side of the lesion, thereby bridging the damaged area to create a functional relay. In addition, the inclusion of a growth factor gradient established by applying neurotrophin-3 (NT-3) at the caudal graft-host interface should facilitate long distance regeneration of host axons. *We hypothesize that this combinatorial approach will create an environment conducive to aligned and efficient motor axon growth, thereby facilitating long distance host regeneration through the graft and/or the establishment of a functional relay by exogenous hES-NSCs.*

Specific Aim 2: Supplementing the Biological Relay with FES

To span the disconnection of brain and movement, we will implant an array of stimulating and recording electrodes spanning from a few millimeters rostral of the injury and graft site to lumbar spinal segments caudal to the injury. This will provide an artificial relay that allows electrical signals to bypass and/or cross the injury site as regeneration proceeds (see Figure). Rostral-to-transection FES electrodes will be placed in the vicinity of the corticospinal tract (CST) to directly stimulate corticospinal axon regeneration [18]. Caudally transected axons undergo degeneration due to loss of somal contact, but alpha motor neurons and interneuronal circuits are maintained, at least transiently, following SCI [27]. FES electrodes will be placed in the vicinity of the ventral horn caudal to the transection to stimulate interneurons and alpha motor neurons. FES caudal to the injury site will be used to maintain muscle physiology and integrity immediately following the loss of descending innervation. Combining the use of stimulating and recording electrodes with the treatments discussed in Aim 1, we hope to both boost long

distance host axonal regeneration through the graft, facilitate the formation of functional relays, while maintaining electrical input to interneurons and motor neurons below the level of injury. *We hypothesize that implantation and utilization of recording and stimulating electrode arrays spanning from T2-L1 will promote long distance host axonal regeneration and/or biological relay circuit formation thereby improving functional locomotor outcomes.*

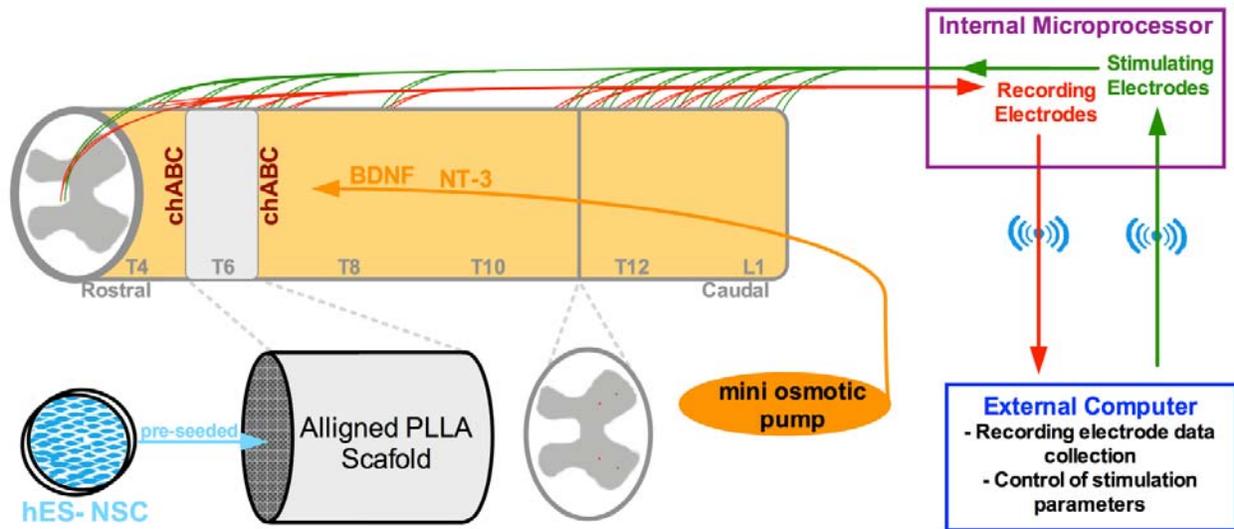


Figure. Schematic diagram demonstrating combination of interventions used in this proposal.

METHODS AND EXPERIMENTAL PLAN

We will first investigate these therapies in a rodent full transection model of SCI before advancing to larger mammals. This model allows us to remove a segment of the cord and replace it with our aligned PLLA matrices [28]. We can then reliably test whether regeneration and functional improvements are taking place. If this preliminary “proof of concept” experiment demonstrates promising results, we will optimize the procedures (i.e., decreasing the invasiveness and update the scaffold and the pumps to the most recent technological advances) and move toward more clinically relevant models of SCI.

Aim 1

Immunosuppressed rats will undergo a full spinal cord transection between T5-T7. Human embryonic stem cells will be pre-differentiated in vitro into neural progenitors (hES-NSC) [25]. The

aligned electrospun PLLA matrix seeded with dissociated hES-NSCs will be implanted acutely into the cavity between T5-T7 and anchored in place with fibrin glue. The matrices will allow axons to grow in straight, parallel bundles and mimic the anatomical organization of the intact spinal cord. In addition, a brain-derived neurotrophic factor (BDNF) and NT-3 loaded mini-osmotic pump will be implanted caudal to the lesion to promote survival of the grafted cells and attract growing axons [29–31]. chABC will be injected at the rostral and caudal portions of the injured cord to prevent the build-up of CSPGs.

Aim 2

Prior to T5-T7 transection, intraspinal microstimulation (ISMS) microwire and sensory arrays will be implanted into the injured spinal cord [23,32]. A number of these arrays will be implanted from T2-T4 in the CST region to locally stimulate this important axon tract. Arrays will also span from T8-L1 within the ventral horn to target interneurons and alpha motor neuron pools. Microwires will be fixed to the dura at their site of insertion, with cyanoacrylate glue drops. Stimulation location and parameters can be mapped by motor cortex stimulation in rodents prior to T5-T7 transection injury. Post transection, the PLLA scaffold will also be implanted (same as Aim 1) with an array of electrodes spanning the transected region. Throughout the post-transection experiment, rostral recordings from descending motor pathways will be sent to a subcutaneous microprocessor programmed to stimulate caudal motor neuron pools. The microprocessor will be implanted subcutaneously and programmed through a wireless connection to ensure proper calibration and allow for adjustments to the rostral recording threshold required to elicit caudal stimulation, as well as the location, timing and intensity of those caudal stimulations.

In vivo locomotor, sensory and spasticity assessments will be performed to monitor the recovery and potential development of sensory allodynia, hyperalgesia and spasticity. Subsequent histological assessments will be performed with particular focus on the survival and neuronal differentiation of grafted hES-NSCs, the integration of the PLLA scaffold with host tissue, and the growth around the graft-host interface. By examining the expression of markers specific to human cells (cytoplasmic antibody or lentivirus carrying green fluorescent protein), we will be able to distinguish between endogenous and exogenous neurons, and thereby assess the relative contributions of long distance regeneration by host

cells versus local relay formation by transplanted cells. Injection of an anterograde tracer, biotinylated dextran amine into the motor cortex 2 weeks prior to sacrifice will allow us to evaluate the extent of axonal sprouting of host cells and visualize the connectivity between host and grafted neurons. Additional analyses will be conducted to look for evidence of aberrant sprouting, long-term damage due to ISMS and recording electrodes embedded in the spinal cord and the possibility for tumor formation.

DISCUSSION AND CONCLUSIONS

Combinatorial approaches are necessary to overcome the lack of functional axonal regeneration and locomotor recovery that occurs following severe spinal cord injury [14]. Therefore, we propose a therapeutic approach that combines a variety of interventions intended to: 1) reduce the inhibitory factors present at the lesion site (chABC), 2) increase the presence of axon growth promoting factors (BDNF & NT-3), 3) replace lost neural connections by transplanting cells capable of generating new neural circuits (hES-NSCs), 4) provide a substrate for neural growth and/or regeneration of appropriate connections (PLLA scaffold), and 5) maintain the excitability of the local and peripheral circuitry involved in motor movements (FES). The FES component supports the locomotor system by keeping it excitable and functioning until axonal plasticity and/or regenerative processes re-establish biologically meaningful connections. Importantly, the FES input can also be altered in response to the output recorded at various levels during the experiment. We believe that this research project represents a required step forward in the study of potential therapies for spinal cord injury, as there is a paucity of work integrating state-of-the-art neuroprosthetic interventions with cutting edge biological approaches. In the present work, a neuroprosthetic implant is envisioned as a tool to help limit the loss of neuronal and muscular function below the level of injury, while potentially boosting plasticity and repair processes involving a combination of biological interventions. Importantly, such a device would allow for continuous monitoring of the ongoing changes in electrical conduction that occur during recovery from spinal cord injury. The availability of monitoring from the electrode array will provide invaluable insight into recovery from spinal cord injury, particularly in terms of the timing and tailoring of interventions to suit ongoing changes in the underlying connectivity of neural substrates.

ACKNOWLEDGMENTS

We would like to thank the organizers the International Symposium on Neural Regeneration (ISNR) and the ISNR Route 28 Summit for giving us the opportunity to be involved in the Route 28 program as well as the many professors that helped mentor and refine our ideas during the course of the ISNR Route 28 Summit. In addition, we thank the editors of the Journal of Rehabilitation Research and Development (JRRD) for giving us the opportunity to publish our proposals. We also thank our individual principle investigators Wolfram Tetzlaff, Warren Alilain, Oliver Raineteau and Jerry Silver.

REFERENCES

1. Sekhon LH, Fehlings MG. Epidemiology, demographics, and pathophysiology of acute spinal cord injury. *Spine*. 2001;26(24, Suppl):S2–12. [PMID:11805601] <http://dx.doi.org/10.1097/00007632-200112151-00002>
2. Dumont RJ, Okonkwo DO, Verma S, Hurlbert RJ, Boulos PT, Ellegala DB, Dumont AS. Acute spinal cord injury, part I: pathophysiologic mechanisms. *Clin Neuropharmacol*. 2001;24(5):254–64. [PMID:11586110] <http://dx.doi.org/10.1097/00002826-200109000-00002>
3. Yiu G, He Z. Glial inhibition of CNS axon regeneration. *Nat Rev Neurosci*. 2006;7(8):617–27. [PMID:16858390] <http://dx.doi.org/10.1038/nrn1956>
4. Fawcett JW. Molecular control of brain plasticity and repair. *Prog Brain Res*. 2009;175:501–9. [PMID:19660677] [http://dx.doi.org/10.1016/S0079-6123\(09\)17534-9](http://dx.doi.org/10.1016/S0079-6123(09)17534-9)
5. Silver J, Miller JH. Regeneration beyond the glial scar. *Nat Rev Neurosci*. 2004;5(2):146–56. [PMID:14735117] <http://dx.doi.org/10.1038/nrn1326>
6. Tuszynski MH, Lu P. Axon plasticity and regeneration in the injured spinal cord. In: Kordower JH, Tuszynski MH, editors. *CNS regeneration: Basic science and clinical advances*. 2nd ed. Boston (MA): Elsevier Academic; 2008. p. 219–335.
7. Brock JH, Rosenzweig ES, Blesch A, Moseanko R, Havton LA, Edgerton VR, Tuszynski MH. Local and remote growth factor effects after primate spinal cord injury. *J Neurosci*. 2010;30(29):9728–37. [PMID:20660255] <http://dx.doi.org/10.1523/JNEUROSCI.1924-10.2010>

8. Kadoya K, Tsukada S, Lu P, Coppola G, Geschwind D, Filbin MT, Blesch A, Tuszynski MH. Combined intrinsic and extrinsic neuronal mechanisms facilitate bridging axonal regeneration one year after spinal cord injury. *Neuron*. 2009;64(2):165–72. [\[PMID:19874785\]](#)
<http://dx.doi.org/10.1016/j.neuron.2009.09.016>
9. Sharma HS. Selected combination of neurotrophins potentiate neuroprotection and functional recovery following spinal cord injury in the rat. *Acta Neurochir Suppl*. 2010;106:295–300. [\[PMID:19812967\]](#)
http://dx.doi.org/10.1007/978-3-211-98811-4_55
10. Karimi-Abdolrezaee S, Eftekharpour E, Wang J, Schut D, Fehlings MG. Synergistic effects of transplanted adult neural stem/progenitor cells, chondroitinase, and growth factors promote functional repair and plasticity of the chronically injured spinal cord. *J Neurosci*. 2010;30(5):1657–76.
[\[PMID:20130176\]](#) <http://dx.doi.org/10.1523/JNEUROSCI.3111-09.2010>
11. Bakshi A, Fisher O, Dagci T, Himes BT, Fischer I, Lowman A. Mechanically engineered hydrogel scaffolds for axonal growth and angiogenesis after transplantation in spinal cord injury. *J Neurosurg Spine*. 2004;1(3):322–29. [\[PMID:15478371\]](#) <http://dx.doi.org/10.3171/spi.2004.1.3.0322>
12. Alilain WJ, Horn KP, Hu H, Dick TE, Silver J. Functional regeneration of respiratory pathways after spinal cord injury. *Nature*. 2011;475(7355):196–200. [\[PMID:21753849\]](#)
<http://dx.doi.org/10.1038/nature10199>
13. Lu P, Tuszynski MH. Growth factors and combinatorial therapies for CNS regeneration. *Exp Neurol*. 2008;209(2):313–20. [\[PMID:17927983\]](#) <http://dx.doi.org/10.1016/j.expneurol.2007.08.004>
14. Ruff CA, Wilcox JT, Fehlings MG. Cell-based transplantation strategies to promote plasticity following spinal cord injury. *Exp Neurol*. 2012;235(1):78–90. [\[PMID:21333647\]](#)
<http://dx.doi.org/10.1016/j.expneurol.2011.02.010>
15. Shapiro S, Borgens R, Pascuzzi R, Roos K, Groff M, Purvines S, Rodgers RB, Hagy S, Nelson P. Oscillating field stimulation for complete spinal cord injury in humans: a phase 1 trial. *J Neurosurg Spine*. 2005;2(1):3–10. [\[PMID:15658119\]](#) <http://dx.doi.org/10.3171/spi.2005.2.1.0003>

16. Hamid S, Hayek R. Role of electrical stimulation for rehabilitation and regeneration after spinal cord injury: an overview. *Eur Spine J.* 2008;17(9):1256–69. [\[PMID:18677518\]](#)
<http://dx.doi.org/10.1007/s00586-008-0729-3>
17. Patel N, Poo MM. Orientation of neurite growth by extracellular electric fields. *J Neurosci.* 1982;2(4):483–96. [\[PMID:6279799\]](#)
18. Carmel JB, Berrol LJ, Brus-Ramer M, Martin JH. Chronic electrical stimulation of the intact corticospinal system after unilateral injury restores skilled locomotor control and promotes spinal axon outgrowth. *J Neurosci.* 2010;30(32):10918–26. [\[PMID:20702720\]](#)
<http://dx.doi.org/10.1523/JNEUROSCI.1435-10.2010>
19. Sadowsky CL. Electrical stimulation in spinal cord injury. *NeuroRehabilitation.* 2001;16(3):165–69. [\[PMID:11790901\]](#)
20. McDonald JW, Liu XZ, Qu Y, Liu S, Mickey SK, Turetsky D, Gottlieb DI, Choi DW. Transplanted embryonic stem cells survive, differentiate and promote recovery in injured rat spinal cord. *Nat Med.* 1999;5(12):1410–12. [\[PMID:10581084\]](#) <http://dx.doi.org/10.1038/70986>
21. Ronaghi M, Erceg S, Moreno-Manzano V, Stojkovic M. Challenges of stem cell therapy for spinal cord injury: human embryonic stem cells, endogenous neural stem cells, or induced pluripotent stem cells? *Stem Cells.* 2010;28(1):93–99. [\[PMID:19904738\]](#)
22. Bamford JA, Mushahwar VK. Intraspinal microstimulation for the recovery of function following spinal cord injury. *Prog Brain Res.* 2011;194:227–39. [\[PMID:21867807\]](#) <http://dx.doi.org/10.1016/B978-0-444-53815-4.00004-2>
23. Bamford JA, Putman CT, Mushahwar VK. Intraspinal microstimulation preferentially recruits fatigue-resistant muscle fibres and generates gradual force in rat. *J Physiol.* 2005;569(Pt 3):873–84. [\[PMID:16239281\]](#) <http://dx.doi.org/10.1113/jphysiol.2005.094516>
24. Blaskiewicz DJ, Smirnov I, Cisu T, DeRuisseau LR, Stelzner DJ, Calancie B. Cauda equina repair in the rat: part 1. Stimulus-evoked EMG for identifying spinal nerves innervating intrinsic tail muscles. *J Neurotrauma.* 2009;26(8):1405–16. [\[PMID:19203211\]](#) <http://dx.doi.org/10.1089/neu.2008.0791>

25. Koch P, Opitz T, Steinbeck JA, Ladewig J, Brüstle O. A rosette-type, self-renewing human ES cell-derived neural stem cell with potential for in vitro instruction and synaptic integration. *Proc Natl Acad Sci USA*. 2009;106(9):3225–30. [\[PMID:19218428\]](#) <http://dx.doi.org/10.1073/pnas.0808387106>
26. Zhang SC, Wernig M, Duncan ID, Brüstle O, Thomson JA. In vitro differentiation of transplantable neural precursors from human embryonic stem cells. *Nat Biotechnol*. 2001;19(12):1129–33. [\[PMID:11731781\]](#) <http://dx.doi.org/10.1038/nbt1201-1129>
27. Gerasimenko YP, Ichiyama RM, Lavrov IA, Courtine G, Cai L, Zhong H, Roy RR, Edgerton VR. Epidural spinal cord stimulation plus quipazine administration enable stepping in complete spinal adult rats. *J Neurophysiol*. 2007;98(5):2525–36. [\[PMID:17855582\]](#) <http://dx.doi.org/10.1152/jn.00836.2007>
28. Yang F, Murugan R, Wang S, Ramakrishna S. Electrospinning of nano/micro scale poly(L-lactic acid) aligned fibers and their potential in neural tissue engineering. *Biomaterials*. 2005;26(15):2603–10. [\[PMID:15585263\]](#) <http://dx.doi.org/10.1016/j.biomaterials.2004.06.051>
29. Tobias CA, Shumsky JS, Shibata M, Tuszynski MH, Fischer I, Tessler A, Murray M. Delayed grafting of BDNF and NT-3 producing fibroblasts into the injured spinal cord stimulates sprouting, partially rescues axotomized red nucleus neurons from loss and atrophy, and provides limited regeneration. *Exp Neurol*. 2003;184(1):97–113. [\[PMID:14637084\]](#) [http://dx.doi.org/10.1016/S0014-4886\(03\)00394-7](http://dx.doi.org/10.1016/S0014-4886(03)00394-7)
30. Zhou L, Baumgartner BJ, Hill-Felberg SJ, McGowen LR, Shine HD. Neurotrophin-3 expressed in situ induces axonal plasticity in the adult injured spinal cord. *J Neurosci*. 2003;23(4):1424–31. [\[PMID:12598631\]](#)
31. Martin Bauknight W, Chakrabarty S, Hwang BY, Malone HR, Joshi S, Bruce JN, Sander Connolly E, Winfree CJ, Cunningham MG, Martin JH, Haque R. Convection enhanced drug delivery of BDNF through a microcannula in a rodent model to strengthen connectivity of a peripheral motor nerve bridge model to bypass spinal cord injury. *J Clin Neurosci*. 2012;19(4):563–69. [\[PMID:22266141\]](#) <http://dx.doi.org/10.1016/j.jocn.2011.09.012>

32. Yakovenko S, Kowalczewski J, Prochazka A. Intraspinal stimulation caudal to spinal cord transections in rats. Testing the propriospinal hypothesis. *J Neurophysiol.* 2007;97(3):2570–74. [PMID:17215510] <http://dx.doi.org/10.1152/jn.00814.2006>

Stem cell derived radial glial cells in magnetically aligned scaffold for repair after spinal cord injury

Justin A. Beller, PhD;¹ Jacquelyn Cragg, MPH;² Zin Khaing, PhD;³ Dylan McCreedy, BS⁴

¹Spinal Cord and Brain Injury Center, University of Kentucky, Lexington, KY; ²International Collaboration on Repair Discoveries (ICORD), University of British Columbia, Vancouver, Canada;

³Department of Biomedical Engineering, University of Texas at Austin, Austin, TX; ⁴Department of Electrical Engineering, Washington University, St. Louis, MO

Abstract—The following was completed as part of the 2011 Route 28 Summit at the International Symposium on Neural Regeneration. The topic of the Route 28 Summit was “Novel Ways to Exploit Stem Cells for Recovery of Human Central Nervous System Function.” Traditional cell transplant strategies have not been successful in promoting robust functional recovery following spinal cord injury (SCI). Part of the reason why cell transplant therapies have failed may be related to improper targeting of regenerating axons through the lesion. In this report, we propose a novel therapeutic approach using stem cell-derived radial glial cells in combination with a magnetically aligned fibrin scaffold for targeted axonal regeneration. Animals with a cervical dorsal column injury will receive an injection of embryonic stem cell-derived radial glial cells along with a fibrin-thrombin solution directly into the lesion. The fibrin scaffold will be aligned in situ using magnetic resonance imaging (MRI) prior to polymerization. To further promote axon growth through the lesion, cyclic adenosine monophosphate and chondroitinase ABC will be co-administered rostral and caudal to the lesion. Functional recovery will be evaluated using electrophysiological assessments and the sticker removal and forelimb reaching tasks. Growth of the regenerating or sprouting host axons will be examined at 3 and 6 mo after injury using MRI and diffusion tensor imaging. Histological analyses will be conducted to visualize the underlying circuitry mediating putative functional recovery. It is hypothesized that the fibrin-mediated linear organization of the radial glial cells in the lesion will enhance targeted axon outgrowth and improve functional recovery following SCI.

BACKGROUND

Radial Glial Cells

Radial glial cells play an integral role in guiding cell migration during development of the central nervous system. In birds and fish, radial glial cells play an essential role in regeneration following injury [1]. Furthermore, data suggest that radial glial cells maintain their phenotype and favor the regenerative response in the presence of a patterned substrate (i.e., fibrin) [2]. Embryonic stem cell-derived cells with a specific radial glial phenotype are readily obtainable [3] (**Figure 1**). Therefore, radial glial cell transplant therapy is technically feasible.

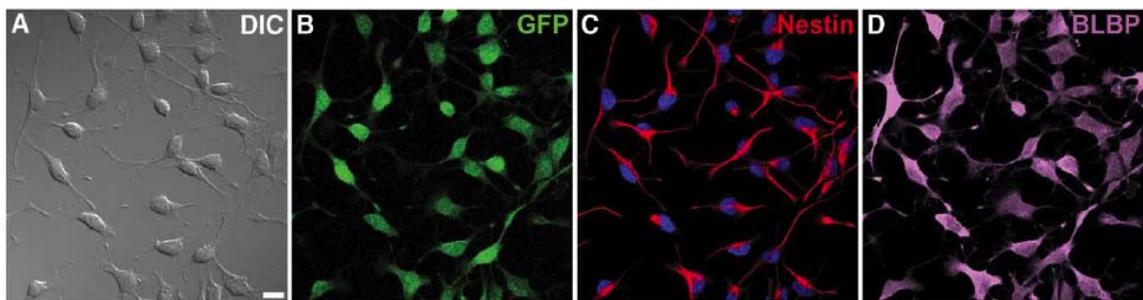


Figure 1. RG3.6 cells showed features of radial glial cells. (a) Differential interference contrast (DIC) and (b) green fluorescent protein (GFP) showing radial glia morphology. Expression of radial glial markers: (c) the intermediate filament protein, nestin, and (d) brain lipid binding protein (BLBP). Scale bar = 20 μm . Adapted with permission from Hasegawa et al. [3].

Aligned Substrates Can Enhance Axonal Growth

It is becoming increasingly evident that physical cues such as topography can play a significant role in guiding axons [4]. For instance, following peripheral nerve injury, Schwann cell basal lamina and the associated extracellular matrix provide guidance cues for the regeneration of axons [5–6].

Specifically, laminin and chondroitin sulfate proteoglycans interact with regenerating axons and either promote or inhibit axonal outgrowth. In a recent study, poly-L-lactic acid microfibers in either aligned or in random configuration were examined to determine the effect topography on axon outgrowth [7]. The authors found that neurites of cultured dorsal root ganglia (DRG) neurons, grown on aligned fibers, reached significantly greater distances compared to randomly aligned fibers and film controls (Figure 2). Moreover, DRG neurons cultured on random fibers produced a denser network of neurites than those

cultured on films without any topography (**Figure 2(a–b)**). However, the overall length of neurites did not significantly differ between these two conditions (random versus control) (**Figure 2(b–c)**), suggesting that the path of neurite growth is more circuitous on randomly aligned fibers. Therefore, while the presence of a suitable substrate is essential, alignment of the substrate can maximize targeted neurite extension by limiting or restricting the direction of axonal growth.

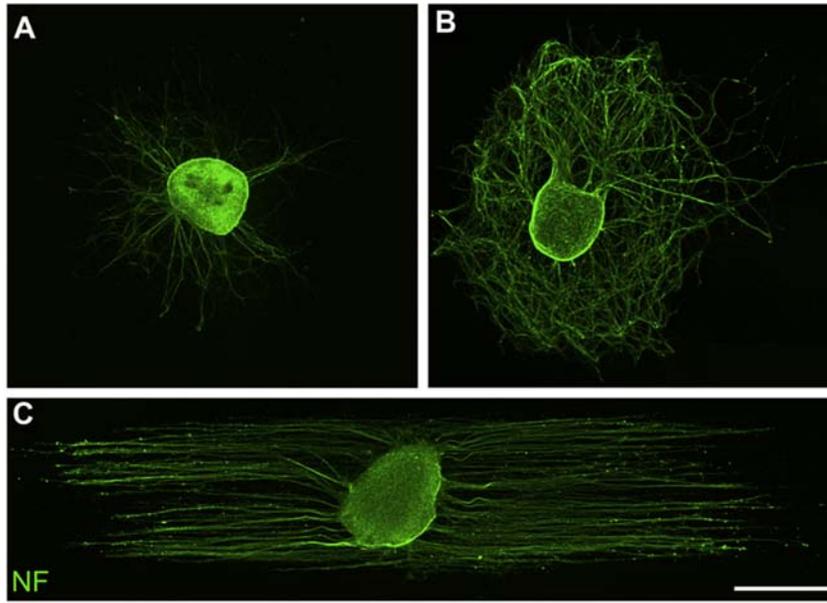


Figure 2. Aligned substrates can enhance axonal outgrowth *in vitro*. Dorsal root ganglia P4 explants were plated onto (a) smooth, (b) unaligned, and (c) aligned fibers. Samples were labelled with neurofilament (NF). Scale bar = 500 μ m. Adapted with permission from Hurtado et al. [7].

Study Proposal

We hypothesize that stem cell derived radial glial cells can significantly enhance axonal outgrowth after injury to the spinal cord. We propose a highly innovative approach that uses MRI to produce an aligned fibrin scaffold.

METHODS

Treatments and Animal Groups

Adult Sprague-Dawley rats will be subjected to a complete bilateral removal of dorsal column between C6 and C7. Animals will recover for two weeks while the injury site stabilizes. Two weeks

following injury, the glial scar will be surgically resected and rats will be injected with embryonic stem cell-derived radial glia (GFP-labeled for better tracking in vivo) in a fibrin-thrombin mixture. Rats will be placed in a MRI scanner to align the fibrin scaffold during a crosslinking reaction with thrombin as previously described [8]. chABC will be injected rostrally and caudally to the lesion site, immediately following removal from the MRI. cAMP will be injected into the C7 DRG to further promote axonal outgrowth. Five experimental groups will be used: radial glia + aligned substrate + cAMP + chABC; radial glia + aligned substrate; radial glia + unaligned substrate + cAMP + chABC; aligned substrate only; and radial glia only.

Histology

At 3 and 6 mo post-injury, the anterograde tracer, biotinylated dextran amine, will be injected into the C7 DRG to assess the extent of axon growth.

Functional Analyses

The sticker removal test for proprioception and reach task for fine motor control, as well as electrophysiological assessments as previously described [9], will be used. The following time points will include behavioral assessments: training (pre-injury), post-lesion, pre-graft, post-graft (with biweekly tests up to 6 mo).

In Vivo Imaging

Longitudinal studies using DTI will be used to visualize the growing axons [10–11].

DISCUSSION AND CONCLUSIONS

It is anticipated that this novel application of radial glial cells will enhance axonal outgrowth and improve functional recovery after SCI. However, a few potential limitations to this approach may exist. We proposed to use fetal-derived stem cells; however, previous studies suggest alternative methods for the development of radial glial cells from embryonic stem cells [3]. The use of embryonic stem cells may have more clinical relevance, due to availability and ethical constraints against the use of embryonically derived cells. Secondly, magnetic alignment of the fibrin scaffold has been successful in vitro but has not yet been attempted in vivo. Therefore, additional modifications may be necessary to obtain

polymerization of fibrin scaffolds in vivo. Thirdly, stability of the fibrin scaffold in vivo may be a limiting factor. Though a possible limitation, previous studies suggest that fibrin gel degradation can be significantly prolonged by either the addition of aprotinin [12] or the addition of polyethylene glycol onto the fibrin [13]. Lastly, if the proposed treatment shows efficacy in this specific injury model, additional testing will be needed to determine if this method can be used in a chronic setting and/or in other injury models.

REFERENCES

1. Peterson RS, Lee DW, Fernando G, Schlinger BA. Radial glia express aromatase in the injured zebra finch brain. *J Comp Neurol*. 2004;475(2):261–69. [\[PMID:15211466\]](#) <http://dx.doi.org/10.1002/cne.20157>
2. Mattotti M, Alvarez Z, Ortega JA, Planell JA, Engel E, Alcántara S. Inducing functional radial glia-like progenitors from cortical astrocyte cultures using micropatterned PMMA. *Biomaterials*. 2012;33(6):1759–70. [\[PMID:22136716\]](#) <http://dx.doi.org/10.1016/j.biomaterials.2011.10.086>
3. Hasegawa K, Chang YW, Li H, Berlin Y, Ikeda O, Kane-Goldsmith N, Grumet M. Embryonic radial glia bridge spinal cord lesions and promote functional recovery following spinal cord injury. *Exp Neurol*. 2005;193(2):394–410. [\[PMID:15869942\]](#) <http://dx.doi.org/10.1016/j.expneurol.2004.12.024>
4. Spivey EC, Khaing ZZ, Shear JB, Schmidt CE. The fundamental role of subcellular topography in peripheral nerve repair therapies. *Biomaterials*. 2012;33(17):4264–76. [\[PMID:22425024\]](#) <http://dx.doi.org/10.1016/j.biomaterials.2012.02.043>
5. Hudson TW, Zawko S, Deister C, Lundy S, Hu CY, Lee K, Schmidt CE. Optimized acellular nerve graft is immunologically tolerated and supports regeneration. *Tissue Eng*. 2004;10(11–12):1641–51. [\[PMID:15684673\]](#) <http://dx.doi.org/10.1089/ten.2004.10.1641>
6. Feneley MR, Fawcett JW, Keynes RJ. The role of Schwann cells in the regeneration of peripheral nerve axons through muscle basal lamina grafts. *Exp Neurol*. 1991;114(3):275–85. [\[PMID:1748202\]](#) [http://dx.doi.org/10.1016/0014-4886\(91\)90153-4](http://dx.doi.org/10.1016/0014-4886(91)90153-4)

7. Hurtado A, Cregg JM, Wang HB, Wendell DF, Oudega M, Gilbert RJ, McDonald JW. Robust CNS regeneration after complete spinal cord transection using aligned poly-L-lactic acid microfibers. *Biomaterials*. 2011;32(26):6068–79. [\[PMID:21636129\]](#)
8. Namani R, Wood MD, Sakiyama-Elbert SE, Bayly PV. Anisotropic mechanical properties of magnetically aligned fibrin gels measured by magnetic resonance elastography. *J Biomech*. 2009;42(13):2047–53. [\[PMID:19656516\]](#) <http://dx.doi.org/10.1016/j.jbiomech.2009.06.007>
9. James ND, Bartus K, Grist J, Bennett DL, McMahon SB, Bradbury EJ. Conduction failure following spinal cord injury: functional and anatomical changes from acute to chronic stages. *J Neurosci*. 2011;31(50):18543–55. [\[PMID:22171053\]](#) <http://dx.doi.org/10.1523/JNEUROSCI.4306-11.2011>
10. Ramu J, Herrera J, Grill R, Bockhorst T, Narayana P. Brain fiber tract plasticity in experimental spinal cord injury: diffusion tensor imaging. *Exp Neurol*. 2008;212(1):100–107. [\[PMID:18482724\]](#) <http://dx.doi.org/10.1016/j.expneurol.2008.03.018>
11. Thuen M, Olsen O, Berry M, Pedersen TB, Kristoffersen A, Haraldseth O, Sandvig A, Brekken C. Combination of Mn(2+)-enhanced and diffusion tensor MR imaging gives complementary information about injury and regeneration in the adult rat optic nerve. *J Magn Reson Imaging*. 2009;29(1):39–51. [\[PMID:19097077\]](#) <http://dx.doi.org/10.1002/jmri.21606>
12. Smith JD, Chen A, Ernst LA, Waggoner AS, Campbell PG. Immobilization of aprotinin to fibrinogen as a novel method for controlling degradation of fibrin gels. *Bioconj Chem*. 2007;18(3):695–701. [\[PMID:17432824\]](#) <http://dx.doi.org/10.1021/bc060265o>
13. Zhang G, Wang X, Wang Z, Zhang J, Suggs L. A PEGylated fibrin patch for mesenchymal stem cell delivery. *Tissue Eng*. 2006;12(1):9–19. [\[PMID:16499438\]](#) <http://dx.doi.org/10.1089/ten.2006.12.9>

Using genetically modified stem cells to halt the progression of ALS

Francisco D. Benavides, MD;¹ Teresa A. Evans, BS, BA;² Todd E. White, PhD;³ Zijia Zhang, BS⁴

¹The Miami Project, University of Miami, Miami, FL; ²Department of Neuroscience, Case Western Reserve University, Cleveland, OH; ³Department of Neurobiology, Morehouse School of Medicine, Atlanta, GA; ⁴Department of Anatomy and Cell Biology, Oklahoma State University, Tulsa, OK

Abstract—The following was completed as part of the 2011 Route 28 Summit at the International Symposium on Neural Regeneration. The topic of the Route 28 Summit was, “Novel Ways to Exploit Stem Cells for Recovery of Central Human Nervous System Function.” Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized by the loss of motor neurons leading to paralysis and death. The vast majority of ALS cases are idiopathic; however, at least 2% are caused by mutation of the copper-zinc superoxide dismutase 1 gene on chromosome 21. Here, we propose a three-pronged approach: (1) identify the molecular trigger for the onset of symptomatic ALS using a microarray approach, (2) develop a genetically modified cell-based treatment, and (3) restore lost respiratory function once disease progression has been halted by an implanted stem cell treatment.

BACKGROUND AND SIGNIFICANCE

ALS is a neurodegenerative disease affecting about 30,000 Americans [1]. The typical timecourse of the disease from onset to death is two to five years. Most cases of ALS are idiopathic and the precipitating factor in genetic cases is yet unknown. Currently, the only approved clinical treatment is Riluzole, which blocks glutamatergic transmission in the CNS [2]. Clinical trials have been conducted with varied success using modified stem cells [3–4], anti-glutamatergic factors [5–7], and neurotrophic factors [8]. Further investigation is needed to determine the cause and molecular triggers of the ALS, and the development of an effective treatment. First, we propose an extensive microarray study using induced pluripotent stem cells (iPSCs) derived from patients with ALS-SOD1 to determine what molecular change occurs at the onset of symptomatic ALS. Second, we propose a novel intervention/therapy using genetically modified autologous hematopoietic stem cells. Finally, we present a simple method for restoring respiratory function in patients using stem cells to form interneuronal relays.

PROPOSED STUDY AND METHODS

Hypothesis Statement

We hypothesize that stem cells can be modified to deliver protective factors to the CNS in order to halt the progression of ALS.

Aim 1: To Determine the Molecular Trigger For Motor Neuron Death And Symptom

Presentation in ALS-SOD1 Patients

We will use gene microarray technology to investigate the gene expression profiles of cells from ALS-SOD1 patients before and after onset of symptoms, and cells from healthy subjects (controls). Fibroblasts will be harvested from ALS-SOD1 patients ($n = 20$) and age matched controls ($n = 5$) every four months over the five year period during which symptomatic onset typically occurs. The fibroblasts will be transformed into iPSCs which will be induced to become motor neurons, oligodendrocytes, astrocytes, microglia and macrophages [9–12]. Since the initiating trigger for ALS is not known, all of these cell types need to be investigated. Mixed cell cultures of all possible combinations of ALS-SOD1 and control cells will be grown. mRNA will be isolated from each culture condition, hybridized to the Affymetrix GeneChip Human Genome U133 Plus 2.0 array for microarray analysis, and fold changes will be calculated. The resulting gene data sets will be further analyzed with Ingenuity Pathway Analysis (IPA, Ingenuity Systems, Inc.) for comparison analysis. Gene expression patterns that correlate with disease progression and cell type will be identified. For this exercise, we hypothesize that we will find transcription regulators that correlate with symptomatic progression. Based on current literature, we propose that these transcription factors will include c-Fos and JunD because the expression levels increase dramatically at the time symptoms are observed [13]. Identifying these molecular triggers will allow for therapeutic interventions that target these molecules and their related signaling pathways.

Aim 2: To Develop Hematopoietic Derived Monocytes Modified to be Protective Against ALS

While Retaining the Innate Ability to Home to Lesioned Areas

In order to deliver therapeutic factors to the sites of neuronal loss in ALS, macrophages derived from autologous bone marrow derived hematopoietic cells by standard protocols [14] will be used to

home to areas of inflammation by endogenous mechanisms. Similar cell types have been shown to home to areas of inflammation in myocardial infarction and glomerular nephritis [15–16]. These cells will then be infected with multiple replication incompetent lentiviral expression vectors to drive the cells toward a wound healing macrophage phenotype, alleviate the damage caused by ALS, and allow for elimination of these cells at later times. Cell lines will then be generated that stably express these factors. Gene expression in all viral vectors will be driven by the MMP-9 promoter.

As presented in the **Figure**, IL4 and IL13 will be used to drive monocytes into an M2 type macrophage phenotype with wound healing properties [17]. To alleviate damage caused by ALS, insulin-like growth factor-1 (IGF-1), somatostatin, c-Jun N-terminal kinase inhibitor (D-JNK-1) and excitatory amino-acid transporter 2 (EAAT2) will be expressed. IGF-1 promotes cellular proliferation, cellular differentiation and inhibition of apoptosis when activated. Although unsuccessful in clinical trials when delivered by subcutaneous injection [18], IGF-1 was shown to exert neuroprotective effects in a mouse model of ALS when delivered by lenti-viral vector [19], and has also shown increases in mesenchymal stem cell engraftment when expressed by transplanted cells [15]. Somatostatin and D-JNKI-1 inhibit c-Fos and JunD, respectively, and, turn off the trigger of ALS that we (hypothetically) derived from our microarray studies [13,20–22]. EAAT2, which increases glutamate re-uptake at the synaptic cleft, will reduce the excitotoxic effect of glutamate in ALS [5,23–24]. Herpes simplex virus-thymidine kinase (HSV-TK) generates monocyte susceptibility to Ganciclovir [25], allowing removal of any excess cells.

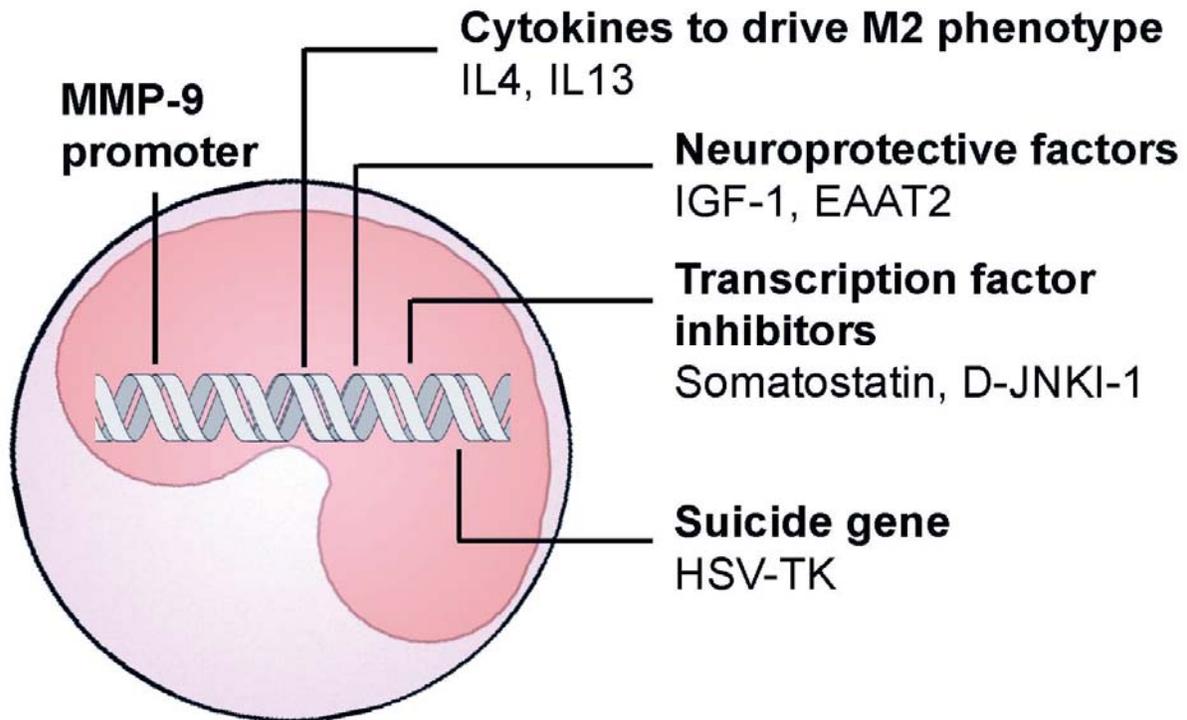


Figure. Proposed genetic modifications of hematopoietic derived monocytes.

We will transfer these modified monocytes into a SOD1-G93A mouse model of ALS using an established femoral vein systemic delivery technique [26]. After transplantation, animals will be monitored. Once symptoms have diminished or stabilized, animals will undergo a blood-brain barrier (BBB) integrity test using IV injection of biotin conjugated dextran [27]. At the point where the dextran is no longer found outside of the blood vessels in sectioned tissue, we will administer Ganciclovir conjugated to a high molecular weight dextran to prevent travel across the BBB and restrict HSV-TK mediated cell death to areas outside of the CNS. In order to prevent excess cell death due to the bystander effect, we will administer dexamethasone concomitantly [28].

Aim 3: To Augment Respiratory Function in a Rodent Model of ALS Once Disease Progression is Halted by Our Treatment Protocol

We will use the SOD1-G93A rat model to test whether autologous bone marrow derived

hematopoietic cells driven to become neural precursor cells (NPCs) [29–30] can promote improved respiratory behavior. NPCs will be stereotactically transplanted in the cervical spinal cord at the level of the phrenic motor nucleus of the transgenic rats. Several segmental injections will be used to deliver cells and populate the area around the phrenic motor neuron pool. NPCs transplanted in similar fashion have been shown to develop into interneuronal phenotypes that become integrated into the phrenic motor pathway and alter respiratory patterns [31–32]. Baseline plethysmographic and electrophysiological parameters will be evaluated and compared to post-transplant time points.

DISCUSSION AND CONCLUSIONS

This proposal describes an innovative approach to understanding and treating ALS. Three challenges are addressed: identification of a precipitating factor in development of ALS symptoms, application of a systemic treatment that will be able to reach the entire CNS in a biologically relevant way, and treatment of the devastating loss of respiratory function seen in late stages of the disease. However, this approach is currently technically unfeasible. First, discovery of the molecular trigger for ALS would require approximately 285,000 microarray chips to analyze all the mixed cell cultures proposed. Such an undertaking would be very expensive and require an enormous amount of labor for tissue processing and data analysis. Allowed unlimited resources, as we were in this exercise, we were freed from this limitation. Second, it is doubtful that a single cell could be stably transfected with as many genes as we have proposed and secrete all these factors at clinically relevant levels. However, this could be approximated with several genetically modified cells being co-transplanted. Transplantation of NPCs to augment respiratory function is feasible but would be insufficient for treating ALS without a treatment to halt or slow the progression of the disease. The idea that transcription factors are the key molecules for the progression of neurodegenerative diseases is being pursued [33] and, therefore, may yet prove to be part of the molecular trigger for symptomatic ALS. Focusing on the factors that lead to progression of the disease instead of the causative factors has the potential to extend the application of these results beyond the SOD1 form of ALS to the idiopathic cases as well.

REFERENCES

1. ALS Association [Internet]. Facts you should know. Washington (DC): The ALS Association; 2010. Available from: <http://www.alsa.org/about-als/facts-you-should-know.html>
2. Doble A. The pharmacology and mechanism of action of riluzole. *Neurology*. 1996;47(6, Suppl 4):S233–41. [PMID:8959995] http://dx.doi.org/10.1212/WNL.47.6_Suppl_4.233S
3. Martínez HR, Molina-Lopez JF, Alez-Garza MT, Moreno-Cuevas JE, Caro-Osorio E, Gil-Valadez A, Gutierrez-Jimenez E, Zazueta-Fierro OE, Meza JA, Couret-Alcaraz P, Hernandez-Torre M. Stem cell transplantation in amyotrophic lateral sclerosis patients. Methodological approach, safety, and feasibility. *Cell Transplant*. Epub 2012 Feb 13. [PMID:22329998]
4. Mazzini L, Fagioli F, Boccaletti R, Mareschi K, Oliveri G, Olivieri C, Pastore I, Marasso R, Madon E. Stem cell therapy in amyotrophic lateral sclerosis: a methodological approach in humans. *Amyotroph Lateral Scler Other Motor Neuron Disord*. 2003;4(3):158–61. [PMID:13129802] <http://dx.doi.org/10.1080/14660820310014653>
5. Kim K, Lee SG, Kegelman TP, Su ZZ, Das SK, Dash R, Dasgupta S, Barral PM, Hedvat M, Diaz P, Reed JC, Stebbins JL, Pellicchia M, Sarkar D, Fisher PB. Role of excitatory amino acid transporter-2 (EAAT2) and glutamate in neurodegeneration: opportunities for developing novel therapeutics. *J Cell Physiol*. 2011;226(10):2484–93. [PMID:21792905] <http://dx.doi.org/10.1002/jcp.22609>
6. Miller RG, Mitchell JD, Lyon M, Moore DH. Riluzole for amyotrophic lateral sclerosis (ALS)/motor neuron disease (MND). *Amyotroph Lateral Scler Other Motor Neuron Disord*. 2003;4(3):191–206. [PMID:13129806] <http://dx.doi.org/10.1080/14660820310002601>
7. Miller RG, Mitchell JD, Lyon M, Moore DH. Riluzole for amyotrophic lateral sclerosis (ALS)/motor neuron disease (MND). *Cochrane Database Syst Rev*. 2012;3:CD001447. [PMID:22419278] <http://dx.doi.org/10.1002/14651858.CD001447.pub3>
8. Saccà F, Quarantelli M, Rinaldi C, Tucci T, Piro R, Perrotta G, Carotenuto B, Marsili A, Palma V, De Michele G, Brunetti A, Brescia Morra V, Filla A, Salvatore M. A randomized controlled clinical trial of growth hormone in amyotrophic lateral sclerosis: clinical, neuroimaging, and hormonal results. *J Neurol*. 2012;259(1):132–38. [PMID:21706151] <http://dx.doi.org/10.1007/s00415-011-6146-2>

9. Amabile G, Meissner A. Induced pluripotent stem cells: current progress and potential for regenerative medicine. *Trends Mol Med.* 2009;15(2):59–68. [\[PMID:19162546\]](#)
<http://dx.doi.org/10.1016/j.molmed.2008.12.003>
10. Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, Yamanaka S. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell.* 2007;131(5):861–72.
[\[PMID:18035408\]](#) <http://dx.doi.org/10.1016/j.cell.2007.11.019>
11. Wernig M, Meissner A, Foreman R, Brambrink T, Ku M, Hochedlinger K, Bernstein BE, Jaenisch R. In vitro reprogramming of fibroblasts into a pluripotent ES-cell-like state. *Nature.* 2007;448(7151):318–24. [\[PMID:17554336\]](#) <http://dx.doi.org/10.1038/nature05944>
12. Ogawa S, Tokumoto Y, Miyake J, Nagamune T. Induction of oligodendrocyte differentiation from adult human fibroblast-derived induced pluripotent stem cells. *In Vitro Cell Dev Biol Anim.* 2011;47(7):464–69. [\[PMID:21695581\]](#) <http://dx.doi.org/10.1007/s11626-011-9435-2>
13. Yoshihara T, Ishigaki S, Yamamoto M, Liang Y, Niwa J, Takeuchi H, Doyu M, Sobue G. Differential expression of inflammation- and apoptosis-related genes in spinal cords of a mutant SOD1 transgenic mouse model of familial amyotrophic lateral sclerosis. *J Neurochem.* 2002;80(1):158–67.
[\[PMID:11796754\]](#) <http://dx.doi.org/10.1046/j.0022-3042.2001.00683.x>
14. Ishikawa K, Yoshida S, Nakao S, Sassa Y, Asato R, Kohno R, Arima M, Kita T, Yoshida A, Ohuchida K, Ishibashi T. Bone marrow-derived monocyte lineage cells recruited by MIP-1beta promote physiological revascularization in mouse model of oxygen-induced retinopathy. *Lab Invest.* 2012;92(1):91–101. [\[PMID:21912378\]](#) <http://dx.doi.org/10.1038/labinvest.2011.141>
15. Haider HK, Jiang S, Idris NM, Ashraf M. IGF-1-overexpressing mesenchymal stem cells accelerate bone marrow stem cell mobilization via paracrine activation of SDF-1alpha/CXCR4 signaling to promote myocardial repair. *Circ Res.* 2008;103(11):1300–8. [\[PMID:18948617\]](#)
<http://dx.doi.org/10.1161/CIRCRESAHA.108.186742>
16. Wilson HM, Stewart KN, Brown PA, Anegon I, Chettibi S, Rees AJ, Kluth DC. Bone-marrow-derived macrophages genetically modified to produce IL-10 reduce injury in experimental

glomerulonephritis. *Mol Ther.* 2002;6(6):710–17. [\[PMID:12498767\]](#)

<http://dx.doi.org/10.1006/mthe.2002.0802>

17. Martinez FO, Helming L, Gordon S. Alternative activation of macrophages: an immunologic functional perspective. *Annu Rev Immunol.* 2009;27:451–83. [\[PMID:19105661\]](#)

<http://dx.doi.org/10.1146/annurev.immunol.021908.132532>

18. Sorenson EJ, Windbank AJ, Mandrekar JN, Bamlet WR, Appel SH, Armon C, Barkhaus PE, Bosch P, Boylan K, David WS, Feldman E, Glass J, Gutmann L, Katz J, King W, Luciano CA, McCluskey LF, Nash S, Newman DS, Pascuzzi RM, Pioro E, Sams LJ, Scelsa S, Simpson EP, Subramony SH, Tiryaki E, Thornton CA. Subcutaneous IGF-1 is not beneficial in 2-year ALS trial. *Neurology.* 2008;71(22):1770–75. [\[PMID:19029516\]](#) <http://dx.doi.org/10.1212/01.wnl.0000335970.78664.36>

19. Kaspar BK, Lladó J, Sherkat N, Rothstein JD, Gage FH. Retrograde viral delivery of IGF-1 prolongs survival in a mouse ALS model. *Science.* 2003;301(5634):839–42. [\[PMID:12907804\]](#)

<http://dx.doi.org/10.1126/science.1086137>

20. Todisco A, Campbell V, Dickinson CJ, DelValle J, Yamada T. Molecular basis for somatostatin action: inhibition of c-fos expression and AP-1 binding. *Am J Physiol.* 1994;267(2 Pt 1):G245–53.

[\[PMID:7915496\]](#)

21. Hirt L, Badaut J, Thevenet J, Granziera C, Regli L, Maurer F, Bonny C, Bogousslavsky J. D-JNK11, a cell-penetrating c-Jun-N-terminal kinase inhibitor, protects against cell death in severe cerebral ischemia. *Stroke.* 2004;35(7):1738–43. [\[PMID:15178829\]](#) <http://dx.doi.org/10.1161/01.STR.0000131480.03994.b1>

22. Hasel C, Dürr S, Bauer A, Heydrich R, Brüderlein S, Tambi T, Bhanot U, Möller P. Pathologically elevated cyclic hydrostatic pressure induces CD95-mediated apoptotic cell death in vascular endothelial cells. *Am J Physiol Cell Physiol.* 2005;289(2):C312–22. [\[PMID:15772124\]](#)

<http://dx.doi.org/10.1152/ajpcell.00107.2004>

23. Gras G, Porcheray F, Samah B, Leone C. The glutamate-glutamine cycle as an inducible, protective face of macrophage activation. *J Leukoc Biol.* 2006;80(5):1067–75. [\[PMID:16912070\]](#)

<http://dx.doi.org/10.1189/jlb.0306153>

24. Liang H, Ward WF, Jang YC, Bhattacharya A, Bokov AF, Li Y, Jernigan A, Richardson A, Van Remmen H. PGC-1 α protects neurons and alters disease progression in an amyotrophic lateral sclerosis mouse model. *Muscle Nerve*. 2011;44(6):947–56. [PMID:22102466]
<http://dx.doi.org/10.1002/mus.22217>
25. Berger C, Flowers ME, Warren EH, Riddell SR. Analysis of transgene-specific immune responses that limit the in vivo persistence of adoptively transferred HSV-TK-modified donor T cells after allogeneic hematopoietic cell transplantation. *Blood*. 2006;107(6):2294–2302. [PMID:16282341]
<http://dx.doi.org/10.1182/blood-2005-08-3503>
26. Paul C, Samdani AF, Betz RR, Fischer I, Neuhuber B. Grafting of human bone marrow stromal cells into spinal cord injury: a comparison of delivery methods. *Spine*. 2009;34(4):328–34. [PMID:19182705]
<http://dx.doi.org/10.1097/BRS.0b013e31819403ce>
27. DiNapoli VA, Huber JD, Houser K, Li X, Rosen CL. Early disruptions of the blood-brain barrier may contribute to exacerbated neuronal damage and prolonged functional recovery following stroke in aged rats. *Neurobiol Aging*. 2008;29(5):753–64. [PMID:17241702]
<http://dx.doi.org/10.1016/j.neurobiolaging.2006.12.007>
28. Robe PA, Nguyen-Khac M, Jolais O, Rogister B, Merville MP, Bours V. Dexamethasone inhibits the HSV-tk/ ganciclovir bystander effect in malignant glioma cells. *BMC Cancer*. 2005;5:32. [PMID:15804364] <http://dx.doi.org/10.1186/1471-2407-5-32>
29. Lepore AC. Intraspinal cell transplantation for targeting cervical ventral horn in amyotrophic lateral sclerosis and traumatic spinal cord injury. *J Vis Exp*. 2011;(55):ii. [PMID:21946609]
<http://dx.doi.org/10.3791/3069>
30. Silani V, Cova L, Corbo M, Ciammola A, Polli E. Stem-cell therapy for amyotrophic lateral sclerosis. *Lancet*. 2004;364(9429):200–202. [PMID:15246734] [http://dx.doi.org/10.1016/S0140-6736\(04\)16634-8](http://dx.doi.org/10.1016/S0140-6736(04)16634-8)
31. White TE, Lane MA, Sandhu MS, O'Steen BE, Fuller DD, Reier PJ. Neuronal progenitor transplantation and respiratory outcomes following upper cervical spinal cord injury in adult rats. *Exp Neurol*. 2010;225(1):231–36. [PMID:20599981] <http://dx.doi.org/10.1016/j.expneurol.2010.06.006>

32. Lane MA, White TE, Coutts MA, Jones AL, Sandhu MS, Bloom DC, Bolser DC, Yates BJ, Fuller DD, Reier PJ. Cervical prephrenic interneurons in the normal and lesioned spinal cord of the adult rat. *J Comp Neurol*. 2008;511(5):692–709. [\[PMID:18924146\]](#) <http://dx.doi.org/10.1002/cne.21864>
33. Kane MJ, Citron BA. Transcription factors as therapeutic targets in CNS disorders. *Recent Pat CNS Drug Discov*. 2009;4(3):190–99. [\[PMID:19891598\]](#)