Combining biological and electrical stimulation therapies following spinal cord injury

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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.*

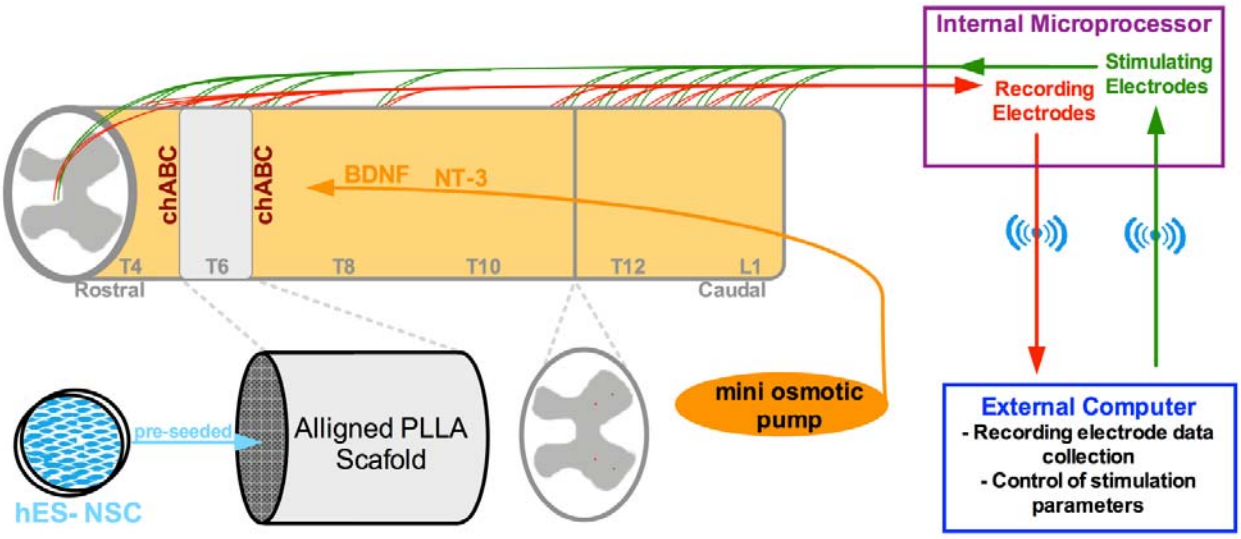

Figure. Schematic diagram demonstrating combination of interventions used in this proposal.

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 florescent 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.

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