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14. ABSTRACT

Spinal cord injury (SCI) exacts an enormous social and financial burden on society. As such, there has been considerable attention directed at finding treatment strategies, including development of tissue and cell transplant techniques. However, the current approaches do not adequately address the complexity of the injury site, such as lesion length and an environment that is usually non-permissive for axon regeneration. We have developed tissue-engineered constructs consisting of living dorsal root ganglia (DRG) and axons that can be stretch-grown to a length necessary to bridge extensive lesions. In current studies, we have optimized in-vitro growth of our constructs, transplanted these constructs into a 1cm-long lesion in the rat thoracic spinal cord, and have demonstrated survival of DRG up to two weeks post-implantation. In addition, we have begun to characterize the in vitro electrophysiological characteristics of the DRG, which may aid in evaluation of synaptic connectivity. In ensuing evaluations, we intend to evaluate long-term (3 and 6 month) survival of the constructs as well as functional recovery beyond the lesion site. If successful, this approach will provide an alternative or additional means to repair large spinal lesions.

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Introduction

There are an estimated 11,000 patients who suffer spinal cord injury (SCI) each year in the US and approximately 200,000 chronic SCI patients[1]. In addition, for US warfighters deployed abroad, SCI has become a common consequence of exposure to blast from improvised explosive devices (IEDs)[2,3,4]. In preparation for this funded grant, we demonstrated proof-of-concept success of bridging a lateral hemisection of the rat spinal cord with engineered ("stretch-grown") living nervous tissue constructs[5]. For the current effort, we have successfully developed a model of 1 cm long complete excision of the thoracic spinal cord. With the new model, we have initiated studies to 1) examine whether the transplanted constructs bridging SCI lesion promote functional recovery, and 2) determine potential formation of new intraspinal circuits across the lesion, such as growth of host axons through the construct and synapse formation with neurons on the other side. Overall, we have been very industrious this first year of funding, hiring and training new personnel, developing and characterizing a new model of SCI and initiating our transplantation studies.

Body

Specific Aim 1: Evaluation of effects of transplanted nervous tissue constructs on recovery of function over 6 months post-injury in a model of complete spinal cord segment excision (T9-T11). We are currently transplanting engineered living nerve constructs consisting of long axonal tracts (1 cm long) to bridge an excised segment (also 1cm in length) of the rat thoracic spinal cord 10 days after injury. Groups include sham (surgery, no injury), injured with hydrogel insertion into the cavity/ lesion, and injured with the living construct embedded in hydrogel transplanted into the cavity. We will perform weekly functional outcome assessments using a battery of tests to evaluate potential recovery of both motor and sensory function.

Specific Aim 2: Evaluation of the survival and integration of transplanted living nervous tissue constructs and host axon regeneration through the construct at 1, 3, and 6 months post-transplantation. Using the same animals/groups from Aim 1, we have begun to perform extensive histological examinations. For our initial analyses, we must determine that the constructs survive for at least 1-2 weeks before studying long-term outcomes. In addition, these initial studies are designed to explore the capacity of the transplanted living constructs to promote the formation of new intraspinal circuits through tract tracing and identification of synaptic integration.

Proof-of-concept: Since the inception of this grant, we have developed a new model to test the efficacy of the transplanted constructs. The removal of bone and excision of the spinal cord spanning three vertebrae poses a great technical challenge for the model. Accordingly, through extensive trial and error surgical development of the model, we have established a novel "trap door" laminoplasty procedure. Importantly, this approach allows re-access to the cavity via the trap door, which, when closed also provides a strong barrier to prevent invasion of connective tissue into the wound (**Figure 1**). Without this laminoplasty, we have found that invading connective tissue completely destroys the transplanted construct.

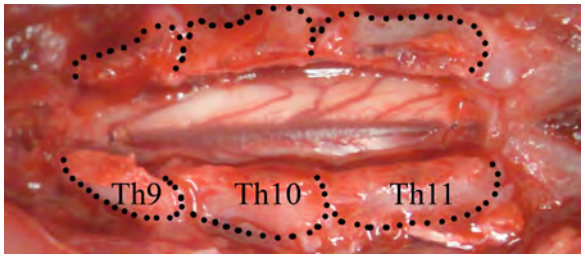

Figure 1. Laminoplastic exposure before injury. A trap-door laminoplasty was created to prevent infiltration of excessive connective tissue in order to preserve the construct. The open leaves of the trap doors are outlined in black dashes.

As expected, complete evacuation of 1 cm of the lateral thoracic spinal cord (from T9-T11; **Figure 2**) induces complete paralysis of the hind limbs (**Figure 3**). This almost extreme SCI design was deliberate so that we will be able to accurately assess temporal functional recovery directly related to transplantation. This lesion also allows ample space for the transplanted construct. Ten days after the injury, 1 cm long nervous tissue constructs (**Figure 4**) are

transplanted into the cavity with the ends proximated with host tissue. This time point was chosen to avoid the immediate post-traumatic inflammation and for a more clinically realistic time of transplant, as opposed to acute repair. To determine the survival of the construct, we have euthanized animals 2 weeks post complete transection and subsequent transplantation. We have demonstrated that clusters of DRG with intact processes survive at this time post-implantation (**Figure 5**).

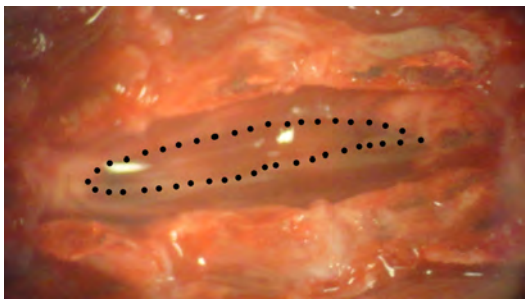

Figure 2. Intraoperative view of complete 10mm transection SCI injury (4 x 10 mm). The lesion is filled with collagen as a control.

Having established that experimental animals in our study with the complete 1cm excision of host spinal cord exhibit complete paralysis, we are currently generating a sufficient "n" in order to proceed with the motor and sensory tests. As opposed to a partial transection or contusion models that often have relatively good functional recovery with no treatment, the complete evacuation of 1cm of spinal cord assures continuing paralysis. Thus, any recovery of function following transplantation with the living construct can be accurately interpreted.

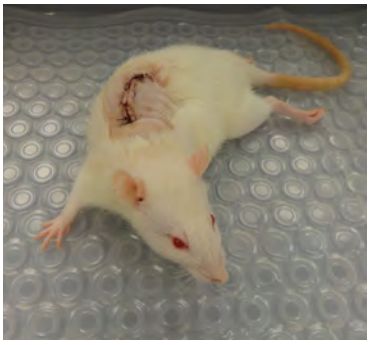

Figure 3. Rat post complete transection SCI injury. No weight bearing on hind limbs and "dragging" of torso occurs due to complete lower limb paralysis.

PITFALLS: During the course of these experiments, we have encountered several obstacles that we are now addressing. Although we have established the general laminoplasty procedure that has greatly improved the model, in some animals we still encounter connective tissue infiltration into the vertebral column. We are rectifying this situation by improving the barrier used to block connective tissue invasion, such as using artificial dura, and an additional barrier with Teflon tape. Additionally, we are assessing the possibility of poorly sealed gelfoam around the laminoplasty.

On the transplant material side, there are no complications with tissue engineering of the construct. Specifically, we routinely produce the transplantable constructs with robust axon tracts spanning two populations of DRG neurons. We are currently attempting to initiate recording the electrophysiological responses from DRG. Our initial efforts have not been successful, potentially due to high impedance. As such, we are in the process of evaluating different electrodes.

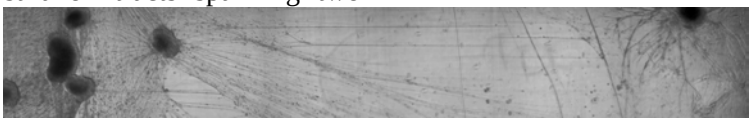

Figure 4. 1-cm dorsal root ganglia (DRG) nerve construct prior to implantation. DRG at each end of the construct extend axons, which are then stretch-grown to a desired length.

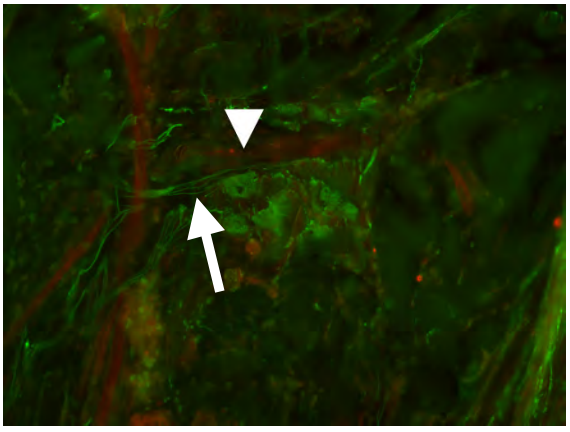

Figure 5. Two week survival of DRG nerve construct in spinal cord. Transplanted DRG bodies (arrowhead) and surviving axons (arrow) identified with SMI31/SMI32 cocktail and visualized with Alexa 488 secondary (green). TRITC (red) merge shows auto-fluorescence.

Key Research Accomplishments

SPECIFIC AIM 1: Evaluation of effects of transplanted nervous tissue constructs on recovery of function over 6 months post-injury in a model of complete spinal cord segment excision (T9-T11).

• TASK: We have demonstrated that the complete thoracic spinal cord transection model of a 1 cm long excision spanning T9- T11 induces complete and permanent loss of function below the lesion. As such, the source of potential recovery of function following transplantation with the living construct will be easily interpreted. In addition, with the complete excision of host spinal cord, tract tracing will also be more readily interpreted.

• TASK: Construct transplantation. We have begun transplantation of constructs in animals that will be maintained for 1, 3, or 6 months post-transplantation end points and survival.

SPECIFIC AIM 2: Evaluation of the survival and integration of transplanted living nervous tissue constructs, and host axon regeneration through the construct at 1, 3, and 6 months post-transplantation.

• TASK: Tissue harvest and histological analyses. We have demonstrated that transplanted constructs survive 2 weeks post-surgery. We are in the process of generating animals that will be sacrificed at the appropriate 1, 3, and 6 months post-transplantation endpoints and processed.

Reportable Outcomes

Due to the extensive model development over this initial year needed to initiate our studies, we have no published reports to note.

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Appendices

PERSONNEL SUPPORT PROVIDED BY THE AWARD

1. Douglas Smith
2. Daniel K. Cullen
3. Kevin Browne
4. Andrew Jaye
5. Timur Litvinov
6. Amy Kim