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TITLE: Development of Practical and Rapid Field-Based Therapies for SCI

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14. ABSTRACT There is a great unmet medical need to develop effective neuroprotective or regenerative therapies for spinal cord injury. Advances in military medicine establish potential opportunities for tailoring interventions for spinal cord injury to the battlefield, where the possibility exist for very early administration of therapeutic substances, followed by more extensive and specialized treatment rendered at a secondary care facility to which SCI victims might be transferred 12-72 hours following an injury. Our proposal is examining whether implantation of allogenic bone marrow stromal cell grafts (BMSCs) into spinal cord lesion site will improve behavior and anatomical outcomes after spinal cord contusion. The BMSCs are genetically engineered to secrete growth factors that we hypothesize will reduce cell lose at the injury site and recruit neuroprotective Schwann cells to lesion site to reduce secondary cell loss cavitation. Positive results of this research could lead to clinical trials.					
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Introduction:

We have designed a set of experiments to test candidate therapies for spinal cord injury (SCI) that could be applied in the battlefield and other acute traumatic settings. Specifically, we aim to optimize a combinatorial treatment to augment behavioral recovery following severe spinal cord contusion. These strategies include: 1) augmentation of the *intrinsic* growth state of an injured neuron by elevating cyclic AMP levels, and 2) modifying the *extrinsic* toxic/inhibitory spinal cord environment by transplantation of cells secreting growth factors. We have identified allogeneic bone marrow stromal cells (BMSC) as potential source for cell transplantation for the following reasons:

- 1) BMSCs obtained from unrelated bone marrow donors can be expanded, genetically modified and banked prior to spinal cord injury.
- 2) Cells can be rapidly thawed and transplanted into a SCI patient during spinal decompression surgery.
- 3) Cells are allogeneic and thus will be rejected by the host after a delay. Delayed cell removal is advantageous because these cells will provide *transient* growth factor expression to minimize secondary injury that occurs days and weeks following the initial injury. In addition, these cells will establish an early scaffold that will support Schwann cell migration into the lesion site, thus providing a late scaffold to potentially support axonal growth.

Specific Aim 1: Establish the Feasibility, Time Frame, and In Vivo Survival of Allogeneic Bone Marrow Stromal Cells.

Specific Aim 2: Examine Anatomical and Behavioral Outcomes of Battlefield-Relevant Treatments After Acute Contusive SCI.

Body:

In the past year we have made substantial progress in the approved Statement of Work. We have transplanted allogeneic marrow stromal cells into sites of acute spinal cord contusion. Upon consultation with neurosurgeons, both civilian and military, we have identified the 2-day post-lesion time point as an appropriate time point for cell transplantation. This is based on: 1) Clinical relevance: the majority of spinal decompression surgeries occur within 2-day post-injury and transplantation of cells at this time would be highly desirable; 2) MSCs do not survive when grafted into a 1-day spinal cord contusion. These BMSCs have been modified to secrete the neurotrophins, brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3).

We performed several experiments in several spinal cord contusion models, including severe thoracic contusions and moderate cervical contusions. Parameters for the engraftment and survival of cells were optimized over successive iterations. We learned that cells are optimally grafted in a fibrin matrix, and that cell survival is good at short-term (1 week) time points, with filling of the lesion site and expression of a reporter gene (Fig 1). As hypothesized in the original grant application, we find that grafted cells

are reabsorbed by a one-month survival time point, with no evidence of residual gene expression. A total of 75 rats were used in these studies in the last year.

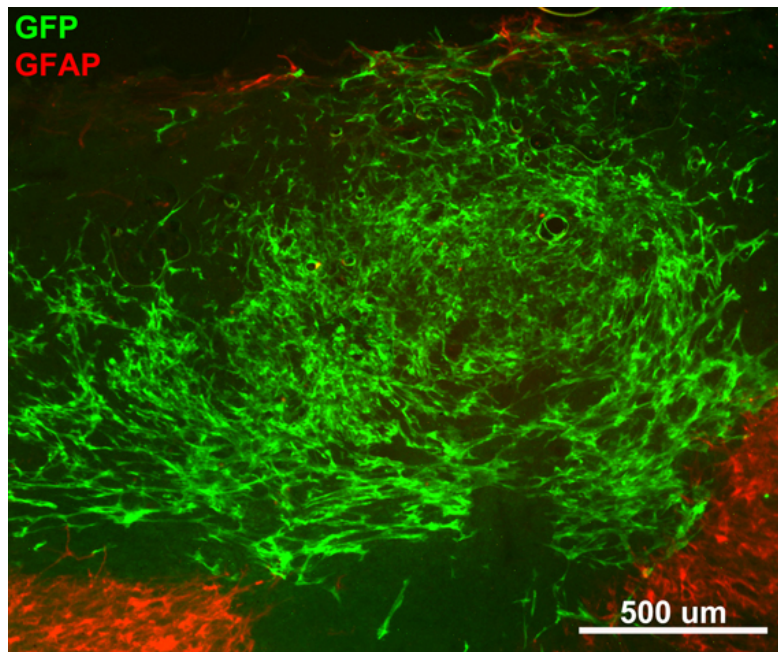


Figure 1: GFP-expressing MSCs embedded in fibrin matrix survive and fill lesion site, defined by GFAP immunolabeling, 7 days post-transplantation. Cells remain present in the lesion cavity for at least 14 days post-lesion.

In the next year, we will apply these advances to specifically determine whether allogeneic bone marrow stromal cells, transduced to express BDNF, result in neuroprotection and improved functional outcomes after severe thoracic contusion injury. The following groups will be examined:

- 1) Lesion only (N = 6)
- 2) Fibrin injected into the lesion site, 2 days post-contusion (N = 6)
- 3) Allogeneic bone marrow stromal cells transplanted 2 day post-contusion (N =12)
- 4) Allogeneic bone marrow stromal cells genetically modified to secrete BDNF transplanted 2 days post-contusion (N =12)

Rats will undergo daily rehabilitation and functional testing over a 3-month post-lesion survival period. Spinal cords will then be perfused and examined for lesion site and axon number below the lesion site. We will label for Schwann cells to confirm their migration and integration into the lesion site, and stabilization/reduction of post-lesion degeneration.

Key Research Accomplishments:

- BMSCs embedded in a fibrin matrix exhibit improved survival compared to grafting techniques lacking a stabilization matrix
- BMSCs embedded in a fibrin matrix survive for at least 14 days following transplantation, and are resorbed by one month post-injury, indicating likely safety for long-term use

- Grafts of BMSCs attract migration and filling of the lesion site by host Schwann cells

Reportable outcomes: In progress.

Conclusion:

Currently, there are no effective treatments for spinal cord injury (SCI). We have identified 2 potential therapeutic targets to limit the amount of secondary injury and to promote behavioral recovery after acute SCI. Cell grafting into a SCI lesion site has well-documented effects on neuroprotection and axonal regeneration. Furthermore, our studies utilize banks of bone marrow stromal cells (BMSCs) obtained from allogeneic donors. These cells provide: 1) a quick and simple cell source that can be transplanted to the injured spinal cord within hours, and 2) a vehicle for transient growth factor expression. Allogeneic cells will eventually be removed by the host immune system, but have been retained for a sufficient time to limit secondary damage and/or promote spinal cord repair. In addition, to cell transplantation, animals will undergo extensive rehabilitation. Weight-supported treadmill stepping and/or swim training have been shown to improve recovery following SCI. We will therefore, combine these two strategies to examine their effects on recovery following acute SCI.

References: None

Appendices: None

Supporting Data:

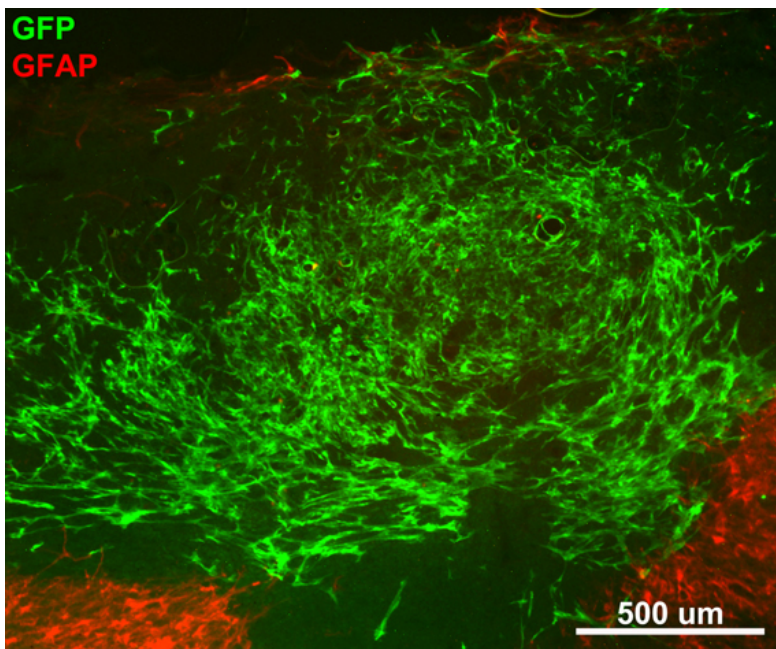


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