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FINAL NARRATIVE REPORT

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Original Specific Aims of the Project

The original aims of the grant application were to assess a novel approach of reconnecting and regenerating the adult rat spinal cord. We had proposed a two-year project to determine whether the adult spinal cord can regenerate when sharply cut and carefully opposed. In the first year, we planned to do 3/4 sections of the spinal cord and determine whether implantation of embryonic astrocytes improve regeneration. In the second year, we proposed to do similar experiments with transected spinal cords, removing a vertebral body to allow the cut ends of the spinal cord to be placed against each other. The funding of the project was cut back significantly from the original requested $100,000 per year for two years to $50,000 for one year.

Project Challenges

We proceeded to initiate the first part of the proposed experiments for the first year, to develop the 3/4 spinal cord section model. In preliminary experiments, we found that a cut of the spinal cord was accompanied by significant fibroblast invasion into the spinal cord, as well as significant subdural and epidural adhesions. The spinal cord surrounding the cut showed marked atrophy. In addition, both subdural and epidural adhesions developed, preventing re-exposure of the spinal cord after 2 weeks. The laminectomy scar adhered so tightly to the dura that it was difficult to re-exposed the spinal cord again. We therefore focused our attention on the solving the problems of repairing the dura to prevent fibroblast invasion into the spinal cord and also preventing both subdural and epidural adhesions. To do so, we first tested several biomaterials for their ability to serve as barrier to prevent epidural adhesions, used the best of these materials in a novel "sandwich" approach to repair the dura, and then carried out 6-week survival experiments to see if these would prevent long-term scarring in rats.

Project Successes

We carried out three sets of experiments on about 100 Long-Evan's hooded rats.

1. Comparison of biomaterials. We first tested three biomaterials for the ability to prevent epidural adhesions. These biomaterials were placed on top of the dura of spinal cords contused with a 25 mm weight drop contusion. At 2 weeks after injury, we examined sagittal sections of the decalcified and paraffin-embedded spinal cords/columns, stained with Mallory trichome stain to examine the extent of epidural adhesions. Specifically, we compared
- a thin (100μ) non-resorbable porous fabric made of polyvinylidene fluoride copolymer fabric made by electrostatic spinning,
- a thin (100μ) resorbable porous fabric made of 60/40 PLA/PCL (polylactic acid/polycaprolactone) absorbable fabric made by electrostatic spinning.
- a thick (120-150μ) non-porous absorbable film of 60/40 PLA/PCL made by compression moding.

2. Long-term prevention of epidural adhesions by PCA/PCL fabric. The experiments showed that the thin resorbable PCA/PCL fabric to prevent epidural adhesions at 6 weeks after spinal cord injury. We discovered that placing a piece of autologous fat on the epidural barrier not only held the membrane in place but prevented formation of the laminectomy scar and kept the barrier in place.

3. Use of thin resorbable PCA/PCL fabric to repair the dura. We initially studied a group of 10 rats with laminectomy, dural opening, and dural repair without spinal cord injury. We developed placed PCA/PCL fabric between the spinal cord and the dural opening, closed the dura, placed another barrier on top. This resulted in a "sandwich" repair of the dura. In 10 rats, the sandwich repair method effectively closed the dura after a 3/4 section of the spinal cord in 80% of the rats. The rats were euthanized at 6 weeks and assessed histologically.

In summary, the thin resorbable PCA/PCL fabric effectively prevented epidural adhesions. The fabric could be placed above and below the dura to "sandwich" the dura and to achieve tight dural closure without suturing or tissue glue, preventing both epidural and subdural adhesions, as well as fibroblast invasion into the cut spinal cord. The surrounding spinal cord showed remarkable little degeneration after a 3/4 section with little atrophy at 6 weeks. We did not see any regeneration across the cut site, suggesting the need to "bridge" the cut in order to achieve regeneration of the cord.

Implications for future research and/or clinical treatment

We have thus developed a effective method of achieving tight dural closure in rats, while preventing epidural and subdural adhesions at the same time. The method prevented fibroblast invasion into the spinal cord after a 3/4 section. This solves several major problems. First, by now, most laboratories that have tried to do chronic cell transplant experiments in the contusion model have probably discovered that severe epidural adhesions develop between the laminectomy scar and the dura after a spinal cord injury. These adhesions are so extensive that the spinal cord laminectomy scar cannot be removed without tearing the dura more than 2 weeks after injury. This may be one of the reasons why there have been few published studies of delayed cell transplantation experiments in rats. Our discovery of a thin resorbable biomaterial that prevents epidural adhesions allows investigators to re-expose the spinal cord for treating chronic spinal cord injury in rats. Second, penetrating wounds of the spinal cord without repair of the dura allow fibroblast invasion into the spinal cord, producing thick fibrous scars. We have developed a novel method of repairing dura by placing thin resorbable biomaterals above and below a dural opening, form a "sandwich". This allows the dura to repair and at the same time prevents both epidural and subdural adhesions. Third, repair of the dura and prevention of fibroblast invasion into the spinal cord resulted in remarkable of the surrounding spinal cord after a 3/4 section.

Plans to continue this research

We theorize that the PCA/PLA fabric prevented epidural adhesion because it was thin, porous, and resorbable. A thin porous non-resorbable fabric made from polyvinylidene fluoride copolymer did not prevent epidural adhesion. However, the laminectomy scar adhered to the PCA/PLA compression molded membrane also did not prevent adhesions, suggesting that it was not the PCA/PLA alone nor the resorbability, that it was a combinaton of restorability and porousness that was responsible. So, we are currently carrying out some experiments to determine whether or not other thin, porous, and resorbable biomaterials besides PCA/PLA will work. We will be seeking further funding to complete the rest of the proposed aims, of using embryonic astrocytes or other transplanted cells to see if they will promote regeneration across the cut interface.

List of Publications

Please see attached manuscript.