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14. ABSTRACT Spinal Cord Injury (SCI)is a serious problem for warfighters injured in far forward areas. Progressive spinal cord tissue swelling secondary to closed trauma aggravate the initial injury. A new low volume resuscitation (LVR) platform using cell impermeant polymers protects systemic tissues after trauma and ischemia by reversing and preventing lethal cell and tissue metabolic swelling. This improves microcirculation and oxygen delivery early after injury. The aim of this project was to test this strategy in SCI in rodents. First, polyethylene glycol (PEG) polymers were sized to optimize use in the spinal cord since the capillary permeability is different. A cocktail of PEG-2k, -5k, and -20k was developed and used in rodent SCI studies. The crystalloid used after injury suppressed tissue swelling and inflammation but failed to affect motor outcomes over 60 days after injury. Return of function of neurogenic bladder was accelerated. Additional dosing, which was deemed needed, produced a study limiting gastroparesis not seen in swine. Although this study was negative, we believe additional doses would produce better outcomes and has potential use in humans.

15. SUBJECT TERMS

Spinal cord injury, tissue swelling, PEG polymers, motor function, rodents, neurotrauma

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1. I**ntroduction**: Spinal Cord Injury (SCI) by itself or associated with complex trauma is a serious problem for warfighters injured in far forward areas. Progressive spinal cord tissue swelling secondary to closed trauma aggravate the initial injury as does the limitations in transport times to hospitals. Stabilization of traumatized tissues in the cord and in other organ systems is necessary to expand the safe pre-hospital and transport times required to improve outcomes for prolonged field stays. A new low volume resuscitation (LVR) platform using cell impermeant polymers protects systemic tissues after trauma and ischemia by reversing and preventing lethal cell and tissue metabolic swelling. This is done by establishing multiple osmotic gradients in the microcirculation to drive water flow directly out of cells and by decompressing the microcirculation to allow more efficient oxygen transfer at very low perfusion pressures and volumes (prevent no-reflow). This dramatically prolongs tolerance to the low volume state, increases safe field transport times, extends survival, and improves outcomes in pre-clinical trauma models. Since similar metabolic swelling mechanisms occur in SCI, it is reasonable to suggest that this platform of IV solutions can be effective for spinal cord swelling too. Therefore, the objective of this study is to identify similar spinal cord specific impermeant PEG polymers, which when added to the existing formulation with PEG-20k, will produce a universal LVR solution effective against complex trauma involving both systemic shock and spinal cord injury in the prolonged pre-hospital battlefield setting.

2. **Keywords**: Spinal cord injury, tissue swelling, PEG polymers, motor function, rodents, neurotrauma

3. **Accomplishments:**

Cerebral Spinal Fluid: One of the initial experiments was to determine the relationship between molecular size and radius of polyethylene glycol (PEG) molecules and the movement and partitioning of these molecules in the microvascular spaces. This experiment was designed to track the relative distribution of labeled PEG tracer polymers of various sizes in the microcirculatory compartments. The goal is to discover a size that does for the spinal cord tissue what PEG-20k does for non-CNS tissues. We therefore need to sample CSF and blood simultaneously in order to determine these partition coefficients. We have discovered that the introduction of catheters into the spinal canal or base of the brain results in instant release of cerebral spinal fluid of enough volume to adequately sample and measure labeled PEG polymers in a scanning fluorescence spectrophotometric plate reader. However, the flow rates over time have proven to be disappointingly low. Therefore, we developed ways to overcome this problem so we can make serial samples over time in the same rat. We tried two options

A.) Do nothing and sample CSF once after administration of the labeled PEG polymer to determine its osmotic reflection coefficient. However, the kinetics of equilibrium in the CNS microcirculation are not known and may be different from what is known in non-CNS tissues. Therefore, it would be nice to get serial CSF samples over 1-2 hours after FITC-PEG injection into the circulation to track the kinetics in a paired design. If this proves too difficult, we will wait 1 hr after injection (a time in non-CNS tissue where equilibrium is reached) and sample the CSF once for measurements.

B.) Try to increase the flow of CSF by infusing saline into the venous side of the circulation after cannulation of the spinal canal. This works for non-CNS tissue to increase lymph flow so it should work for fluid generated across the brain capillaries too.

We developed a hybrid approach where we infuse saline intravenously at a rate that is known to cause diffusion independent movement of solutes across the capillary membrane in peripheral beds (by solvent drag transfer only) and then we samples once after a 15 min equilibration period after delivery of the fluorescently labeled PEG test polymer. This technique produced consistent reproducible measurements of polymers partitioned between the CSF and the plasma that showed size dependency. This technique was deemed better than introducing chronic catheters in the small spaces, which produced tissue injury per se, likely altering the results.

Spinal Cord Injury Model: Before we can begin testing the effects of various sizes of PEG polymers on the development and progression of spinal cord injury after contusion, we need to describe the injury before testing the polymers (positive control). Therefore, we have begun conducting weight drop contusion experiments in rats not receiving any treatments. This validates our injury model, establishes a baseline to test against our experimental polymer groups, and trains the technical staff in conducting the model with ease, reliability, and repeatability. Results are shown in the figure.

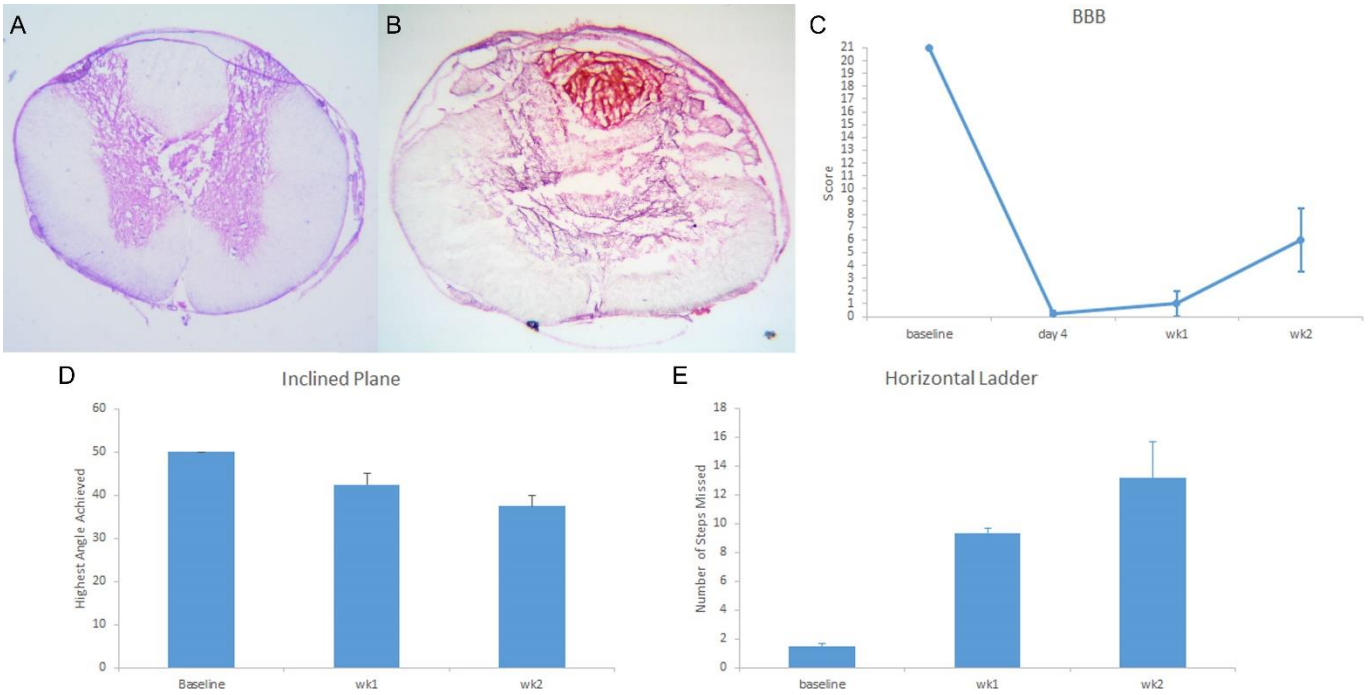
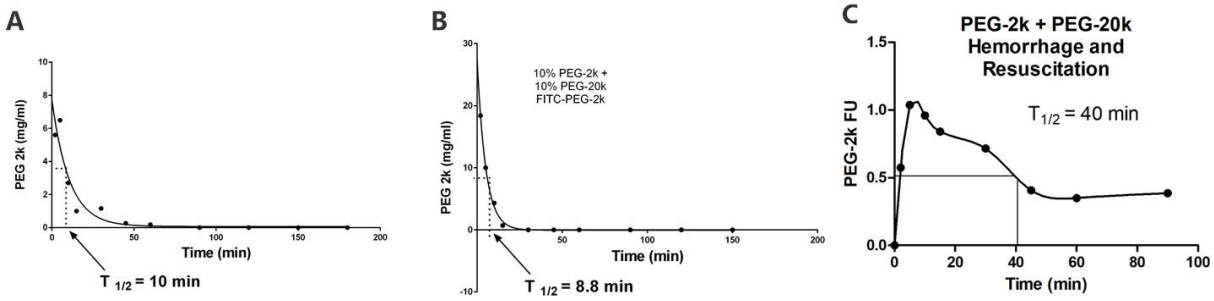


Figure 1: Spinal Cord Injury Optomization.
Fig 1A is a photomicrograph of an intact (uninjured) spinal cord from an adult male Sprague Dawley rat, stained with haemotoxylin and eosin, while Fig. 1B shows a spinal cord contusion site one week following injury, using the NYU MASCIS (New York University, Multicenter Animal Spinal Cord Injury Study) impactor. A 10g rod was dropped from 12.5mm high (12.5mm/sec) onto the spinal cord. Behavioral testing shows prior to injury rats perform well on the open field assay (BBB assay: Fig. 1C), can stabilize themselves on the inclined plane to the maximum angle (50 degrees to the horizontal; Fig. 1D), and have minimal mis-steps on the Horizontal Lader (Fig. 1E). Four days after injury, the rats show poor ability to move their hindlimbs in an open field (BBB), but this improves by week 2, as does their ability to stabilize on the inclined plane. On the horizontal ladder rats appear to have worse performance (more hindlimb mis-steps) on week 2 compared to week 1, however at week 1 they are dragging their hindlimbs, while by week 2 they are using their hindlimbs to step, which causes more misteps than week 1.

<u>Polymer Pharmacokinetics (PK)</u>: Since the optimal PEG polymer size is considerably smaller than the usual PEG-20k, the pharmacokinetics will necessarily be different and needs to be known. The smaller size allows the molecules to traverse the capillaries in the spinal cord tissues but also allows the molecules to escape into Bowman's space in the glomerulus of the kidney nephron, where it becomes trapped in the tubular filtrate (because the molecule is cell impermeant). This means it will be eliminated in the urine and may potentially draw tubular water with it acting as an osmotic diuretic. Furthermore, a reduced half-life may alter its efficacy in injury states, because it is around less. To measure the peak blood levels and half-life of elimination of PEG-2k polymers, we injected rats IV with FITC-labeled PEG-2k and followed plasma levels over time as well as urinary levels. Additionally, we examined the PK of PEG-2k at 1% combined with PEG-20k at 10%, because that will be the formulation given to animals with polyytrauma (both hemorrhagic shock and spinal cord injury). The PEG-20k is needed to cause cardiovascular resuscitation and restart oxygen transfer in the microcirculation while the PEG-2k is the CNS-specific polymer size that was just demonstrated to be effective in the spinal cord (as measured by its optimized reflection coefficient. The PK data are shown in Figure 2.

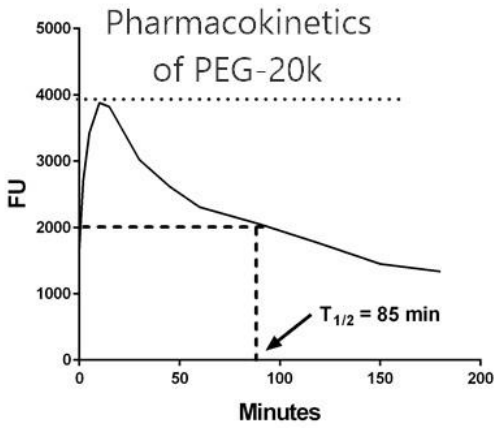


PEG-2k alone (panel A) and with PEG-20k (panel B) is shown in Fig 2. Clearly, the half-life of elimination is very short, on the order of 10 minutes. This value is not altered when it is combined with PEG-20k either. The bulk of the material that is disappearing from the plasma over time was found to appear in the urine, as expected (data not shown). This means that the therapeutic effective time for this polymer to move water out of the swollen spinal cord tissues is short. However, since the renal filtration potential in shock is different than with a healthy cardiovascular system, we repeated the elimination curves in 2 animals that were shocked and undergoing

resuscitation with a mixture of PEG-20k (10%) and PEG-2k (1%). The PEG-2k was tracked by adding a small amount of tracer PEG-2k labeled with FITC. The shocked state dramatically increased the half-life of the PEG-2k (panel C), as expected, because renal filtration is lower or absent in shock and hypotension.

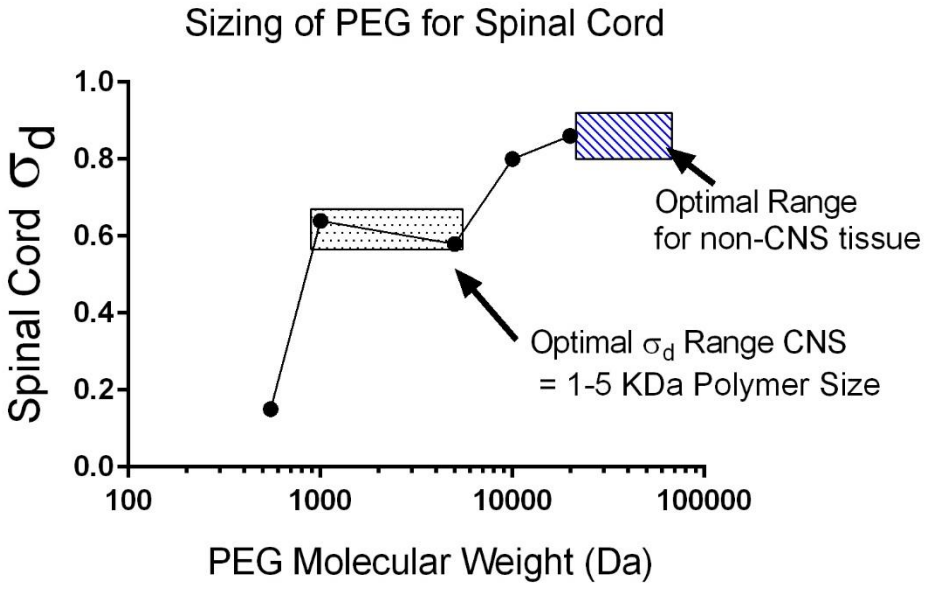
In conclusion, PEG-2k was found to be the best size for establishing favorable osmotic gradients for water movement in metabolically swollen spinal cord tissues. The half-life of the 2k polymer is much less than that for PEG-20k (Figure 3), but the half-life is dramatically increased in the shocked state, probably due to lower renal filtration in the shocked state.

FIGURE 3



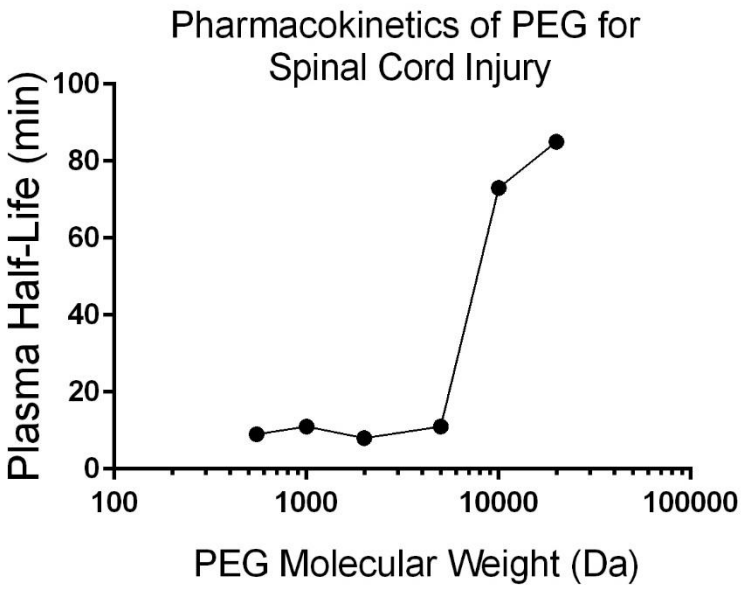
Polymer Sizing and Microcirculatory Excursions in Spinal Cord Tissue: A complete characterization of the osmotic reflection coefficient of all possible active polymer sizes of PEG below 20 kDa was characterized. The goal was to identify polymers that possess the intermediate σ_d having a value of about 0.4-0.6 to ensure the microcirculation in the cord tissue has the optimal distribution of solute molecules to affect rapid water transfer in traumatized tissue. Figure 4 shows these results using specially designed fluorescently labelled PEG polymers ranging in size from 550 to 20,000 Da.

Figure 4



There is established a clear relationship between the molecular weight of the polymer and the σ_d. Remembering that the higher the σ_d, The less permeable the molecule is and the less it partitions into the interstitial space. A value of 0 indicates free permeability and equal equilibration on both sides of the capillary wall while a value of 1.0 indicates completely impermeant behavior such that all of the polymer stays in the capillary. PEG polymers with a size between 1,000 and 5,000 have an acceptable osmotic reflection coefficient (about 0.6). Polymer sizes smaller than 1000 are to permeable and those over 5,000 are not permeable enough. As a reference, PEG-20k was characterized and found to be too big. This size polymer is optimal in non-CNS tissues but clearly may not be optimal in damaged spinal cord tissue. Therefore, these studies clearly show an optimal range of 1-5 kDa polymer size for all future studies in spinal cord injury. For polytrauma studies where water pull from both CNSNS tissue is required, we will test a new LVR solution that contains both PEG-20k (optimized for non-CNS tissue) and a polymer of 1-5 kDa (optimized for CNS tissue).

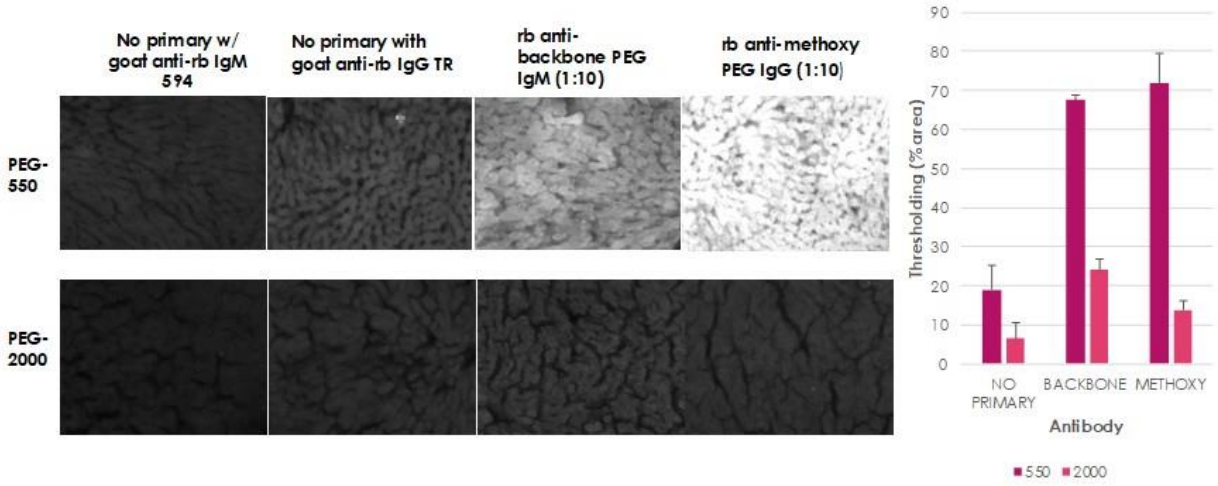
Polymer Sizing and Pharmacokinetics for Spinal Cord Injury: Pevious studies in our lab demonstrated renal clearance of PEG-20k when used for low volume resuscitation. Because PEG is mostly biologically inert, it is excreted mainly unchanged in the uring by filtration across Bowman's space. Once in the renal tubules, the polymer is not reabsorbed because it is impermeant to the apical membrane of the tubular epithelium, analogous to mannitol. One exception is the excretion into the urine by tubular epithelium by reverse pinocytosis from the basolateral membrane. But this route is slow and accounts for the elimination of low concentration residual PEG molecules and most of the material is filtered and excreted. Since size generally influences filtration in the glomerulus, we characterized the half-life of elimination of the spectrum of PEG polymers studied to determine if there was a relationship between size and clearance. Using fluorescently labeled tracer PEG polymers, we were able to track PEG blood levels over time after an IV bolus infusion. These data are seen in **Figure 5**:



Clearly, smaller size polymers have a shorter T1/2 than larger polymers. The sizes optimal for microcirculatory distribution determined in the earlier studies have a much shorter half-life than PEG-20k. Thus will be a consideration when formulating a dosing strategy in animal with spinal cord injury. However, two other factors will alter these results that have not yet been modeled and tested. Specifically, the combination of the small PEG with a larger PEG may increase the half-life of the smaller PEG and the smaller PEG half-life will necessarily be increased in shock and trauma where the renal function is significantly depressed. We are currently modeling those two forces on the pharmacodynamics of small PEG elimination. Obviously, factors that serve to keep the polymer in the circulation longer will mean a longer and more effective biological response to swelling after spinal cord injury. We also hypothesize that short duration is OK in CNS injury because we likely only need a short time to rapidly draw small amounts of water out of the spinal canal space. Once removed, the PEG-20k in the LVR solution should hold the water outside so the later disappearance of the smaller polymer may by then be moot.

PEG Immunohistochemical Localization Protocols: We identified two commercially available antibodies directed against polyethylene glycol polymers that had some validation in the literature. One antibody was directed specifically at the backbone polymer ethylene glycol repeat units in the PEG polymer. The other antibody was raised against the methoxy group of methoxy-PEG, which is a chemical derivative of PEG where a terminal methyl alcohol group is attached to the end of the polymer. To test these antibodies, rats were injected IV with either methoxy PEG with molecular weights of 550 Da or 2,000 Da. After an hour, liver and spinal cord tissues were recovered for immunohistochemical staining using the two antibodies. Liver was used as a positive control where we know these polymers will distribute based on our other work. The main objective was to see which antibody worked better so we removed the tissue of origin as a variable by starting with the staining of liver sections. The sections were prepared at 30 uM thickness on a cryostat and then permeabilized and stained with primary antibody followed by a fluorescently labeled secondary antibody directed against the rabbit IgG primary antibody. The sections were then visualized and photographed using fluorescence microscopy. In some control slices, the primary antibody was omitted to identify non-specific binding in the tissue as a background so the antibody specific staining could be determined. The results of these experiments is shown in Figure 6.

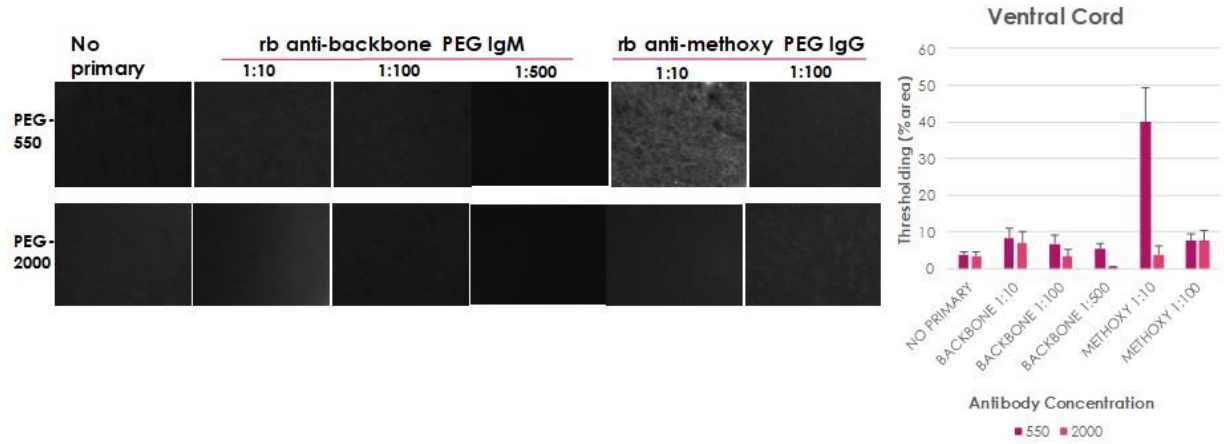
Figure 6



As can be summarized by the bar graph to the right in the figure, both backbone directed and methoxy directed primary antibodies produced significant signal above the background from the secondary antibody alone. Furthermore, the signal intensity was much greater for the smaller 550 polymer compared to the much larger 2000 Da polymer, which is consistent with the known partitioning of these sizes in tissues. The smaller 550 polymer size is a cell impermeant and distributes to the interstitial space whereas the larger 2000 Da polymer is less permeable to the sinusoids in the microcirculation. The main conclusion is that both commercially available primary antibodies to PEG work for staining PEG in tissues. Furthermore, the exact protocol steps that were used to stain the tissues also worked. We will next try this protocol and the two antibodies on spinal cord tissue in the rat.

We next repeated the previous studies but in both brain and cord tissue from uninjured rats. These results are shown in Figure 7 for cord tissue using both backbone and methoxy antibody at various titers. Again, some sections without the primary antibody were included as a control for nonspecific staining.

Figure 7.

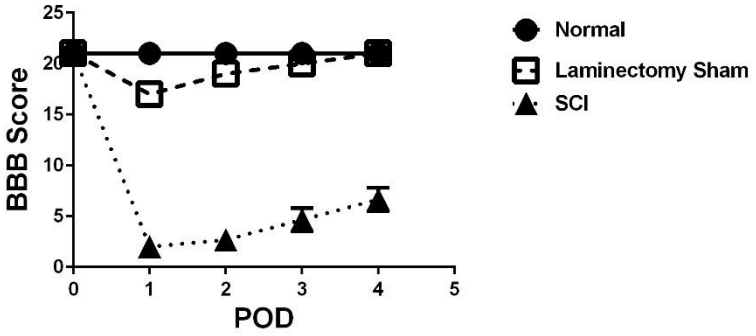


Unlike the liver tissue, which produced strong specific binding signals for both 550 and 2000 Da PEG polymers, only the small 550 was detectable in the neuronal tissues from the CNS. However, since we know that the antibodies are capable of detecting 2000 Da PEG in tissues, these results mean that either 2000 is not getting into the spinal tissue or the antibody is not optimized to see it if it is there. From our previous work measuring osmotic reflection coefficients in the cord, we do know that PEG polymers as large as 5000 are able to escape the capillary and enter the cerebral spinal fluid. Therefore, we conclude that the most likely cause of not seeing 2000 PEG in the cord tissue is because the antibody binding is not optimized. Because we will use PEG polymers in the cord specific flush that are sized bigger than 550 (probably between 2000-5000 Da), we must increase the sensitivity of the assay to be able to detect these larger polymers in the cord tissue. We will spend some time on this as we move to the cord injury and solution-testing phase of the study. We believe that optimization of an immunohistochemical detection approach to determining PEG polymer distribution will be superior to using the fluid compartment labelling experiments that we performed in year 1 to study differential polymers distribution analysis in injured spinal cord tissues.

Spinal Cord Injury Experiments: In some of the early control spinal cord injury animals without any treatment, locomotor function was graded using the Basso-Beattie-Bresnahan (BBB) locomotor rating scale. The BBB scale uses a range from zero (no hind limb joint movements) to 21 (normal movements and coordinate gait). Before surgery, all animals are handled in the openfield maze once-daily for 7 days preceding surgery. On the first postoperative day (POD1), and at PODs 4, animals are placed in the open field and

observed for 4 min. At each POD time point, all animals per group are tested. Two researchers blinded to the treatment group observed the animals in open-field testing. Hind limb movement scores were averaged to obtain a single score for each animal per time point. Mean BBB scores are tallied and plotted as a function of time after injury. The scores of a few rats are shown in Figure 8 (mean +/- SD).

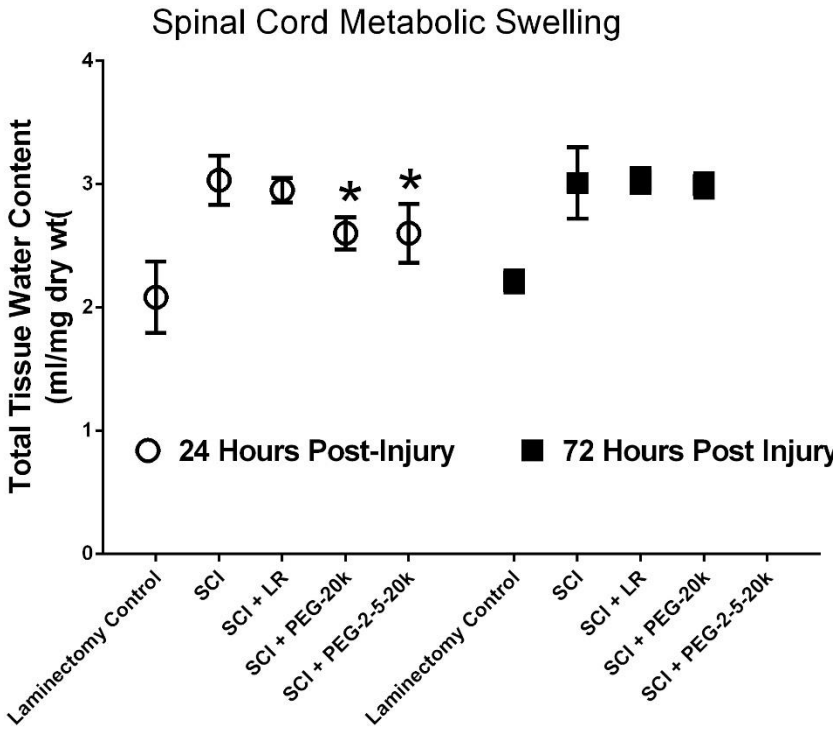
Fig 8



Normal rats without surgery and surgical sham rats that underwent a laminectomy without impact injury scored normal on the BBB movement test, where a score of 21 is considered normal. A slight deficit in the laminectomized sham rats is seen in the first 2 post-operative days due to movement irregularities secondary to incisional pain and muscle detachments /scarring during the laminectomy period. Clear differences are seen in the rats with spinal cord injury (SCI) from impactor lesions created on the opened spinal cord compared to either the normal rats or the laminectomized rats. The SCI injured rats slowly regain movement over the first 4 post-operative days. These control injury scores will be used later to assess the effects of the prototype impermeant solution given to the rats shortly after spinal cord injury.

Metaboloic Cell Swelling with Spinal Cord Injury: In earlier studies, we determined the osmotic reflection coefficients of a variety of sizes of PEG polymers, with the goal of finding ones that have optimal water transfer properties to move water out of metabolically swollen cord tissue after injury. After identifying smaller polymers (2-5 kDa) as optimal, we began testing their water pulling power in injured spinal cord tissues by measuring total tissue water (TTW) content of the spinal cords after injury. The results of these studies is summarized in **figure 9**. Rats were given a standardized SCI injury protocol and the spinal cord tissue was removed 24 or 72 hours later for determination of TTW content. Some animals were not injured but underwent laminectomy only and some were injured and received either LR (lactated ringers solution) or PEG-20k dissolved in LR, or a cocktail of PEG-2, -5, and -20k in what we believe is an optimized LVR solution.

Figure 9.



We documented a 30% increase in tissue swelling or tissue water content after 24 hours after injury. The LR vehicle did not affect this degree of swelling but both LVR solutions containing PEG significantly reduced tissue swelling as indexed directly by measuring total tissue water content. Interestingly, there wasn't a benefit with the "optimized" PEG polymer cocktail compared to PEG-20k, which is used for hemorrhagic shock. Also of interest was the loss of effect after 72 hours from injury where the spinal cord tissue regained water weight equal to what was observed on day 1 in the untreated rats. While the treatment effect with PEG appears small, this probably reflects logarithmic decreases in intra-canal pressure and increases in spinal cord microcirculatory blood flow. This may not be identified under the experimental conditions used because a laminectomy was performed to expose and injure the cord. We speculate that cord swelling, pressure development, and drops in capillary blood flow from microcirculatory compression would be much higher in normal field conditions where a laminectomy is not performed that allows the spinal tissue to freely swell outside of the closed case of the spinal canal. Finally, we hypothesize that combining spinal cord injury with hemorrhagic shock in our polytrauma experiments will potentiate these effects further since PEG-20k has powerful effects during shock alone to increase capillary perfusion. It is probable that hypovolemic shock and SCI potentiate injury to the cord and that the PEG treatment effect will be much greater under those real-life conditions.

The return of swelling after 72 hours could be related to two events. The swelling may be metabolic in nature and reflect the still injured neurons ability to actively control their own cell volume or the later swelling may not be metabolic in nature and it may represent a secondary inflammatory response that causes capillary leak from inflammatory mediators. This extracellular swelling (edema) is different from intracellular metabolic swelling. Histological assessments are ongoing to determine the cause of this secondary swelling, which will help us understand if a second round of impermeant dosing may be effective and worth trying.

Short Term Locomotor Function in SCI:

In other studies, we began to examine motor outcomes in rats with SCI in the following groups

1.) Controls with just laminectomy surgery (no SCI)
2.). Rats with SCI
3.) Rats with SCI treated with the LR vehicle I.V.
4.) Rats with SCI treated with PEG-20k dissolved in LR, and
5.) Rats with SCI treated with a cocktail of PEG polymers, including PEG-2k, PEG-5k, and PEG-20k.

Before and at various days after spinal cord injury, rats were tested using the Basso-Beattie-Bresnahan (BBB) locomotor rating scale. The BBB scale uses a range from zero (no hind limb joint movements) to 21 (normal movements and coordinate gait). Before surgery, all animals are handled in the open-field maze once-daily for 7 days preceding surgery. On the first postoperative day (POD1), and at PODs 4, 7, 10, 14, and 21, animals are placed in the open field and observed for 4 min. At each POD time point, all animals per group are tested. Two researchers blinded to the treatment group observed the animals in open-field testing. Hind limb movement scores are averaged to obtain a single score for each animal per time point. Mean BBB scores are tallied by injured groups and plotted as a function of time after injury. **Figure 10** shows some very early results in 2-3 rats per group.

Figure 10.

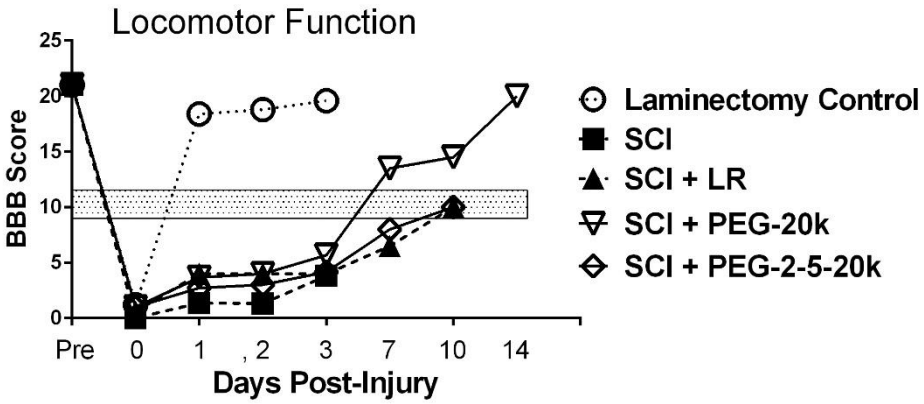
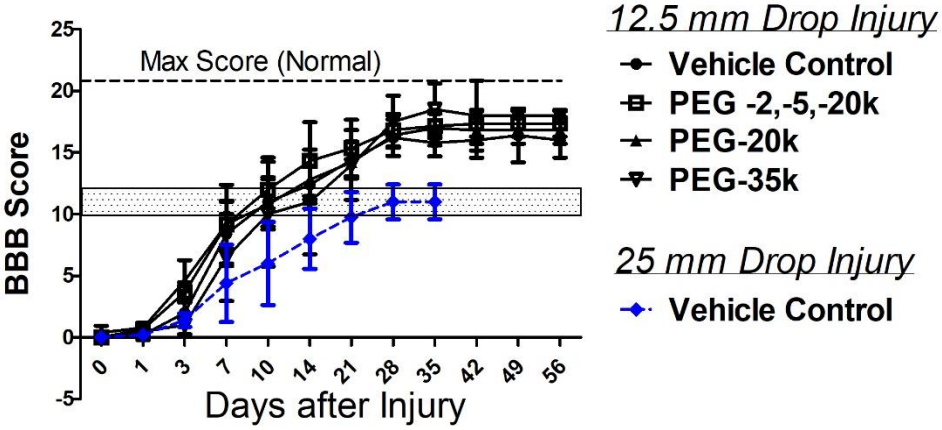


Figure 10 shows the score of control rats with just surgery and no SCI. They initially score very low on the day of surgery due to anesthesia and from muscle pain and discomfort secondary to the laminectomy, which is common to all groups. But, the controls rapidly regain motor control and return quickly to baseline function in 1-2 days after surgery. By contrast, the SCI alone rats show clear deficits in motor activity over 10-14 days. Generally, these animals historically will not get better after 10-14 days and their score of 8-12 on the BBB is about as good as it will get. This plateau 10ange of scores is shown in Figure 5 by the hatched box area. The SCI rats receiving a single low volume resuscitation of 10% PEG-20k so far are doing better on this test starting at 7 days after injury, relative to the untreated groups. So far, it seems that the PEG-20k is able to provide higher levels of motor recovery of the hind limbs. More studies need to be done for longer time periods to be sure. Interestingly, the cocktail of small and large PEG polymers, which we believed may be better than PEG-20k alone, seems to show no improvement over the untreated controls.

Long term locomotor function in SCI: The results of the 8-week testing (8 weeks following initial spinal cord injury) in 5 groups of rodents is shown in **figure 11**.

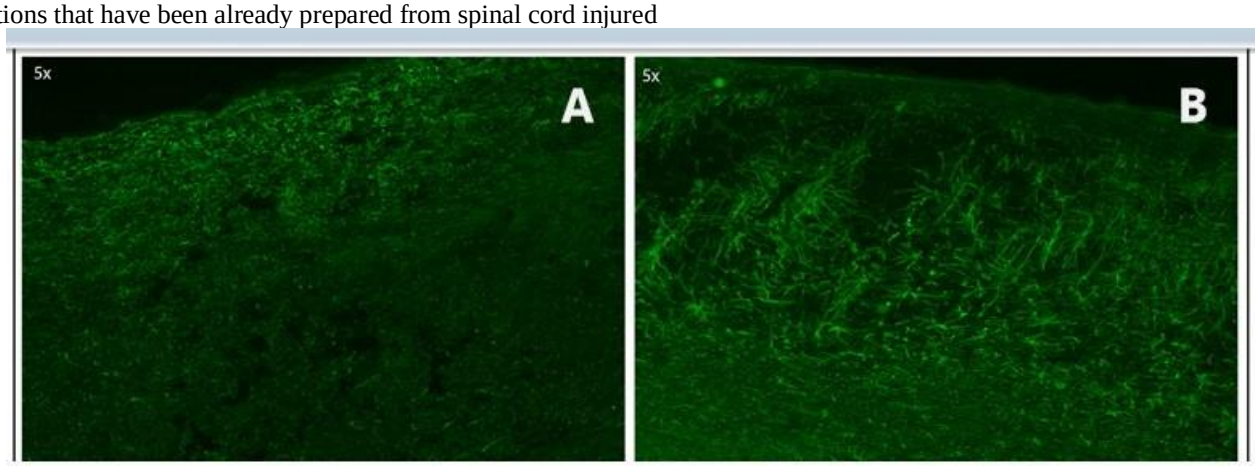


Our initial studies used an injury severity of a 12.5 mm drop height. This means that the open spinal cord was impacted by the SCI device impactor traveling downward onto the spinal cord from a height of 12.5 mm under the force and acceleration of gravity. This is the typical impact severity setting and the most used in the literature. In reported studies using the 12.5 mm severity level, the rats start with almost complete paralysis of the cord after one day from impact but slowly regain motor function of the cord over 4 weeks to achieve a steady

state plateau just below about 50% of normal on the BBB scale, or about a score of 10 (Fig 6). However, all of our groups showed an almost complete return of cord function after 4 weeks. Not surprising, we did not see any differences in any of the treatment groups using the 12.5 mm drop height, probably because there was no room for improvement in the model because even the vehicle treated rats fully recovered. The reason why our results are less severe compare to others reported in the literature is not clear. The technician operating the injury device does so consistently but many subjective factors can explain differences in outcomes, even when the drop height remains unchanged. Specifically, the alignment of the stopping point of the impactor on the cord and the depth it is allowed to penetrate the cord (0.5 mm) can be subjective and result in different outcomes between operators. We posit this is what happened since we use the same technical operator for all of our SCI injury studies in this project. Therefore, in an attempt to improve the resolution of the BBB assay to be able to detect changes in treatment groups, we increased the drop height to 25 mm to lower the scores that the control rats plateau at in order to provide room for improvement, should it occur. In early studies in a separate group of injured rats receiving only the LR vehicle with an injury severity of 25 mm, we observe a plateau more consistent with other studies. Five rats have been taken out to 5 weeks post-injury and show a plateau of function reflected by a BBB score of 10-11 (Figure 6, blue curve). The hatched area is where we would like to be based on historical results. As we finish taking these rats out to 8 weeks, we feel confident now that we will be able to see improvements in spinal cord function in any treatment groups using the higher severity model, should improvements actually occur.

Neurobiological evidence of SCI in 25 mm drop model: The major outcome in this model is spinal cord function as indexed by locomotor function in various tests as the rats recover after spinal cord injury. The trajectory of their recovery tells us if the new resuscitation strategies will be effective for attenuating SCI injury. These motor function curves take 2 months to performs so a lot of time and resources are invested in each rat. Therefore, we want to be certain that the exact model used is performing as it is intended to perform. Therefore, in this year, we have invested a lot of time into validating the 25 mm weight drop model, as used by our lab, will produce the anticipated outcomes in the control rats. We seem to have good motor function results on the BBB test. We also are establishing the histological and immunohistochemical outcomes that historically predict the severity of the injury. We are in the process of now analyzing all of the spinal cord tissue using these tests. **Figure 12** shows the early results from a few rats. These data clearly demonstrate the anticipated anatomic changes associated with the injury site and the associated neuro-inflammation that follows, which all confirm our model severity. This gives us confidence when we move into the third year where multiple test groups will be compared to the control groups in both chronic spinal cord function testing (motor function tests) and histological outcomes.

Figure 12: Frozen sections of the rat spinal cord 72 hours after injury with immunohistochemical staining for Glial Fibrillary Acidic Protein (GFAP). GFAP is a CNS specific intermediate filament cytoskeletal protein and marker for activate astrocytes. Expression of this protein indicates the degree of inflammatory reaction to the cellular injury. Both spinal cord sections below show a large degree of GFAP expression at the site of impact injury. Panel B is from a control rat resuscitated with lactated ringers solution and panel A is from a rat resuscitated with 10% solution of PEG-20k, 5k, and 2k. The resuscitation was performed 30 minutes after spinal cord injury was induced with a 25 mm weight drop. The early analysis from a few rats in each group seems to suggest a much larger change in GFAP expression in the control compared to the PEG treated rats. Much more analysis needs to be performed on countless more sections that have been already prepared from spinal cord injured



rats.

Polytrauma model development- SCI plus Hemorrhagic Shock: A major goal of this project is to determine the role of PEG polymer on spinal cord injury outcomes in both SCI alone and SCI with co-existing trauma and shock because that is likely the most common clinical manifestation of SCI in military field medicine and because the two injuries will necessarily potentiate one another. To that end, we have developed a polytrauma shock protocol using our standard rat shock protocol with modifications to accommodate an SCI insult as well. The requirements of this model is that it produce the greatest **survivable** traumatic and metabolic injury as possible. Survivability is key because the SCI injury component requires chronic development and monitoring. Preliminary studies show progress on a new model modified from the original lethal model by adjusting downward the amount of oxygen debt delivered during hemorrhage. We moved the lactate value that would trigger resuscitation from 10 mM to 7 mM. This requires an average blood loss of about 30% of the estimated total blood volume of the rat. The model nicely produces a severe metabolic and cardiovascular derangement that is 100% survivable on its own and after a spinal cord injury using a 25 mm weight drop model.

Figure 13 below shows the arterial pressure response in the two models after the saline low volume resuscitation is administered to the rats but before a spinal cord injury is induced.

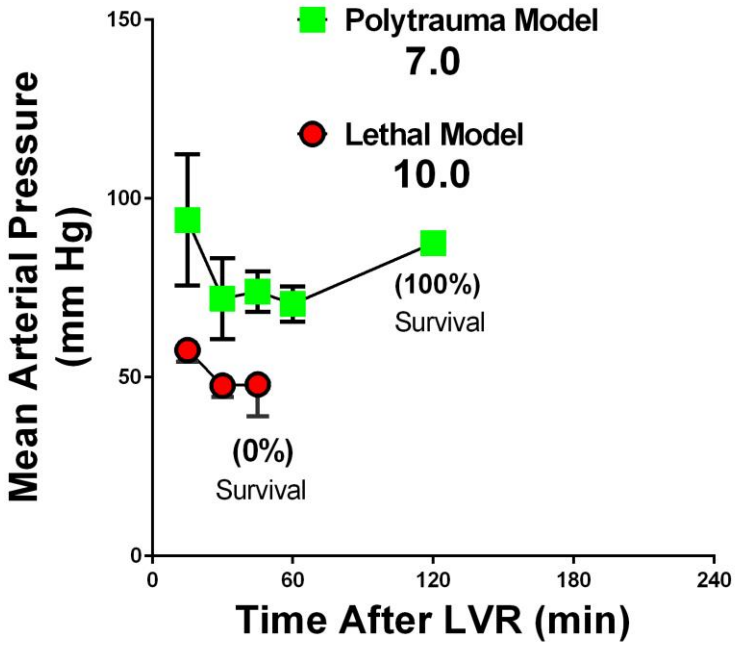
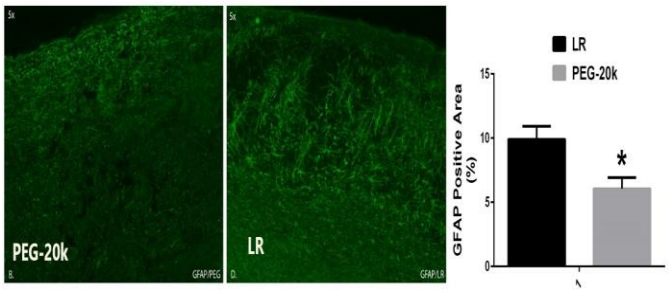


Figure13: Two shock models developed for studies using newly developed PEG polymer based crystalloid LVR solutions. The polytrauma model will be used in this study to assess the effects of both trauma and weight drop on spinal cord injury and motor outcomes in the rats and how early field resuscitation with PEG polymers can alter these outcomes.

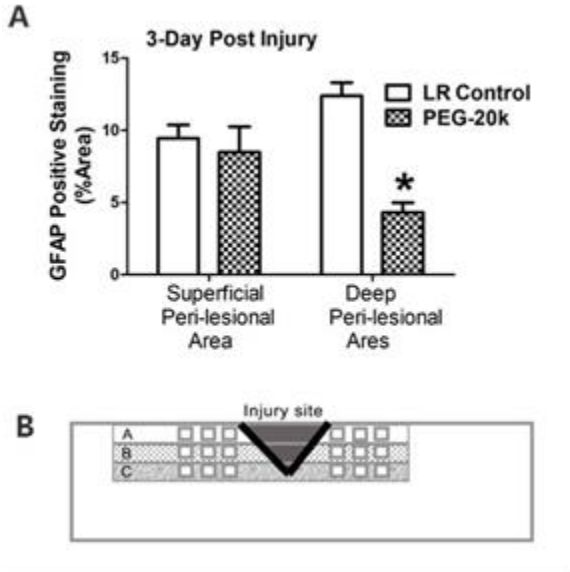
SUMMARY: In the first year we characterized the theoretical optimum polymer size for achieving the best result in moving water out of the metabolically swollen spinal cord after injury. In this current year, we have focused on documenting the model behavior in the control animals so we can be able to visualize changes in the treatment groups, should they occur. We also recalibrated the shock component of the polytrauma model that will allow us to do testing on, not only spinal cord injury per se, but also spinal cord injury in an animal with co-existing cardiovascular and metabolic trauma (shock) in a clinically relevant polytrauma model. Finally, we have shown that the rat spinal cord tissues are behaving normally following injury by their astrocyte reactivity (inflammatory injury). This establishes a firm baseline in all of our models to now test the efficacy of an optimized polymer solution on neurological injury to the cord and on cord motor function as the animals recover from spinal cord injury.

Astrocyte Reactivity: An objective in this year was to examine the effects of spinal cord injury using this newer model on astrocyte reactivity to injury and the effects of PEG polymer treatment. We used expression of Glial fibrillary acidic protein (GFAP) to index injury activation since astrocytes in the spinal cord will increase expression of GFAP in response to mechanical injury in SCI. If PEG-20k administration in the early hours after spinal cord injury is maintaining local tissue oxygenation and preventing lethal metabolic cell and tissue swelling, we hypothesized that a secondary effect may be to reduce local astrocyte injury and GFAP expression. We tested whether neuronal inflammation following injury in spinal cords could be altered and attenuated by the use of PEG-20k in injured rats. To that end, we examined the expression of Glial Fibrillary Acidic Protein (GFAP) in spinal cord astrocytes. Cortical sections were prepared from perfused whole brain specimens without regard to specific areas. This common protein is expressed in injured areas by surrounding astrocyte glial cells and is easy to detect in the perilesional areas. We examined expression of this protein in spinal cord of rats injured with the 25 mm drop model for those receiving either LR control or PEG-20k. The results are shown in **Figure 14**.



The antibody targeting GFAP is fluorescently tagged with FITC so higher expression results in higher area of fluorescence. The injury-induced expression of GFAP is clearly reduced in cords treated with PEG-20k compared to cords treated with the same volume of the lactated ringers solution vehicle. We don't know if the lower inflammation may contribute to better cord function or if other mechanisms of PEG-20k cause less tissue injury that naturally results in less inflammation. We believe it is the later because we present strong evidence that PEG treatment reduces metabolic cell swelling after injury, which is the known primary mechanism of action of this solution.

We next tested whether neuronal inflammation in deep Vs. superficial perilesional areas following injury in spinal cords could be altered and attenuated by the use of PEG-20k in injured rats. To that end, we examined the expression of Glial Fibrillary Acidic Protein (GFAP) in spinal cord astrocytes. We examined expression of this protein in spinal cord of rats injured with the 25 mm drop model for those receiving either LR control or PEG-20k cocktail administered at the optimal dosing shown in figure 15. These regional GFAP results are shown in **Figure 15**.



The data (panel A) clearly shows a very strong anti-inflammatory effect of the PEG optimized solution compared to LR solution in deep perilesional areas (panel B, sections C) compared to more superficial perilesional areas (Panel B, section A). These results suggest that areas around the injury site that are not directly damaged also show inflammatory reactivity and glial activation, which spread like an outward wave from the injury site to affect much larger portions of the injured regions. Most interesting was the strong effect PEG-20k treatment had on the activation of these deep perilesional glial cells, probably in the hypocampus, since it reduced the inflammatory response by about 70%. We are hoping that this translates into better spinal cord motor function and faster return to normal function over time in treated patients.

Spinal Cord Motor Function Testing:

Early Spinal Cord Function Assay:

The main motor outcome assay for this project is to use repeated measurements of spinal cord function, motor activity, and movement behavior by using the Basso, Beattie, and Bresnahan (BBB) motor score. However, this assay requires 2 months of animal study after spinal cord injury and requires considerable time and resources. Since we have evidence that small changes in polymer solution composition and dosing could have very significant differences in tissue swelling and water transfer outcomes, we chose to optimize the solution and dosage to maximize motor function recovery after spinal cord injury. However, since the BBB assay requires months, we decided to use "return to bladder function" as an early marker of later spinal cord motor function in the chronic BBB assay. In this model, as occurs clinically, spinal cord injury is associated with both loss of motor function distal to the injury as well as loss of neurogenic bladder function. As the cord undergoes healing and repair, bladder function returns slowly and predicts the future changes in improvements in spinal cord motor function elsewhere. Therefore, we used the rate of return to bladder function to index later changes in spinal cord function to efficiently and iteratively test our solution compositions and dosage.

TABLE: *Secondary IV polymer dosage and composition testing scheme given after spinal cord injury and after the initial first full dose of PEG-2,-5,-20k solution administered at a volume dose of 10% estimated blood volume (EBV). In this particular trial, all repeated doses were given IV every 4 hours for 24 hours starting 4 hours after the initial loading dose of polymer (10% EBV).*

Repeat Dose Concentration (% EBV)	LR Control	PEG -2, -5, -20k	PEG -5, -20k
0%	X	---	---
1.25%		X	
2.5%		X	X
10%		X	

The composition of each solution is as follows

PEG-2, -5, -20k

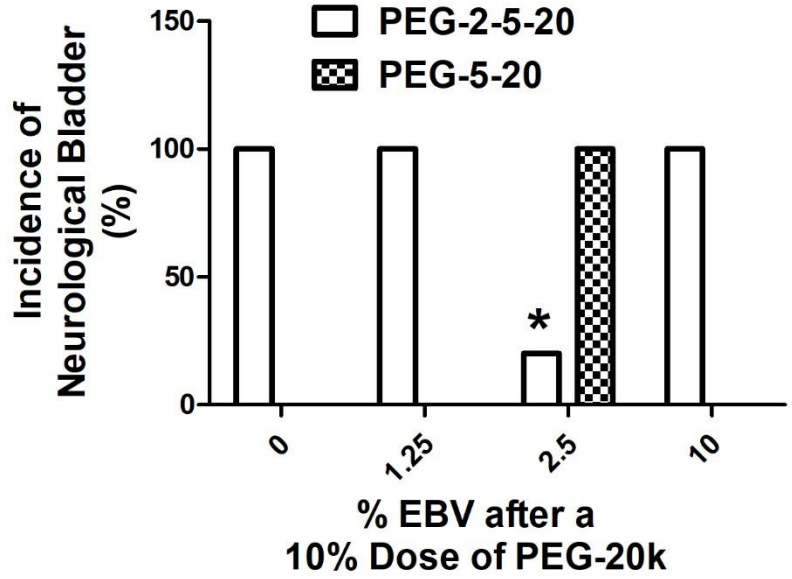
- 100 g PEG-20k/liter
- 25 g PEG-5k/liter
- 10 g PEG-2k/liter
- Solvent base is Lactated Ringers (LR) solution

PEG-5, -20k

- 100 g PEG-20k/liter
- 25 g PEG-5k/liter
- Solvent base is LR solution

Protocol: Adult rats underwent spinal cord injury using the sever 25 mm drop model as described previously. This model produces a motor deficit starting at 100% hindlimb paralysis for the first 3 days followed by a progressive recovery of function to about a 50% maximum level of activity after about 3-4 weeks. Neurogenic bladder is 100% in rats for the first 10 days when recovery slowly resumes after then. The rats must be manually "urinated" each day since their bladder muscles don't contract due to interruption in neural input from the spinal cord injury. Immediately after drop injury and while the rats are still anesthetized, a loading resuscitation of polymer solution (10% PEG-20k containing 2.5% PEG-5k and 1% PEG-2k) is administered at a dose equal to 10% of the estimated blood volume (typically about 2.2 ml). The resuscitation is administered IV over about 5 minutes and the venous catheter is left in place for further infusions. The incisions are closed and the rats are allowed to awaken and given post-operative narcotics for pain and antibiotics. For this period, we studied 3 solutions and 4 dosing regimens. One solution was LR as a control and the other two were variants on the smaller PEG polymers added to the 10% PEG-20k. The dosage was always given at 4 hour intervals over 24 hours since that was determined to be the best for maintaining therapeutic blood levels and it is thought that therapeutic blood levels over 24 hrs may produce maximum neurological benefits. One solution version was missing PEG-2k since we though this size may produce toxicities. The dosages tested of the repeat solutions were 0%, 1.25%, 2.5%, and 10% of the estimated blood volume (EBV), given every 4 hours. After the 6 total doses were given, the cannula were recovered and the rats were allowed to remain for 3 days, after which they were euthanized. The primary outcome in this series (a finding assay design) was rate of return to bladder function as indexed by urine spots in the cage and by an index to quantify the size of the rats bladder each day, twice per day over the 3 day observation period. A summary of the major findings for the early neurological results are shown in Figure 16 for bladder function

Figure 16.



There is a clear benefit and a maximal spinal cord early function present when a cocktail of PEG-20k-2k-5k is administered at repeated q4 dosing at a dose of 2.5% estimated blood volume (about 0.5-ml per dose). Again, all trials used an initial loading dose after injury of 10% EBV. Volume doses higher or lower were not effective and loss of the 2k polymer from the cocktail abolished the therapeutic benefit seen in the 2.5% dose. To be clear, these data show that ***the optimized dosing and cocktail produced no incidence of neurogenic bladder after injury in 4 out of 5 animals***! Since 80% of these animals were able to have normal bladder function immediately after spinal cord injury, we believe that this specific dosing regimen will produce best improved long term spinal cord motor function in the chronic BBB test. Conversely, all other rats not treated in this way had 100% and complete bladder dysfunction after injury for the first 3 days. Therefore, we will now conduct long term surgical recovery trials in the BBB test and rotorod test in rats treated with the optimized solution (PEG-20-2-5k) and dosage (2.5% EBV, q4 x 24 hrs) and compare these outcomes to the lactated ringers volume control or a mannitol control. We believe that this form of early acute testing narrowed the possibilities for successfully testing in the more clinically relevant BBB test.

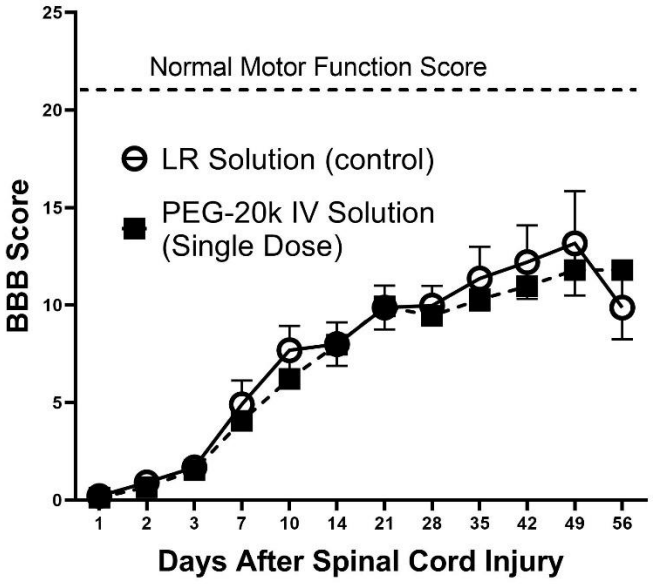
Basso, Beattie, and Bresnahan (BBB):

A major activity was to determine the motor function of rats after spinal cord injury with and without PEG polymer treatment using the Basso, Beattie, and Bresnahan (BBB) scoring system. The severity of the test was increased by using a 25 mm weight drop compared to the previously used 12.5 mm drop. This was done because all spinal cord injured animals fully recovered motor activity in the 12.5 mm drop. Typically, testing is performed with a severity model sufficient to leave all or most of the animals with about a 50% motor deficit that is not reversible in the control group. Assessment of locomotor behavior is an important measure of long-term functional recovery after SCI, and serves as an important tool for evaluating the therapeutic efficacy of the impermeant polymer delivery. The 21-point open field locomotion score was developed by Basso, Beattie, and Bresnahan (BBB) in order to study the sequence of locomotor recovery patterns takes into consideration the early (BBB score from 0 to 7), intermediate (8-13) and late phases (14-21) of recovery (Basso et al., 1995). A score of 21 is normal and a score of 0 is complete paralysis. Any treatment which may increase the BBB scores may be considered neuroprotective. It is generally believed that the peak score at the plateau is important

rather than the rate of change of function. We tested SCI injured rats receiving the lactated ringers control solution (vehicle) and a cocktail of PEG-20, -2, and -5K polymers because the smaller sized molecules displayed the theoretically optimal partition coefficients in the microcirculation of the CNS. We administered the polymer once since the repeated dosages caused significant gastric paresis. This prevented the rats from eating and drinking regularly and complicated survival and performance on the BBB test. Animals underwent laminectomy, and received spinal cord injury using a 25 mm hit. Animals were survived for 56 days. The Basso, Beattie, and Bresnahan (BBB) motor scores used are described below.

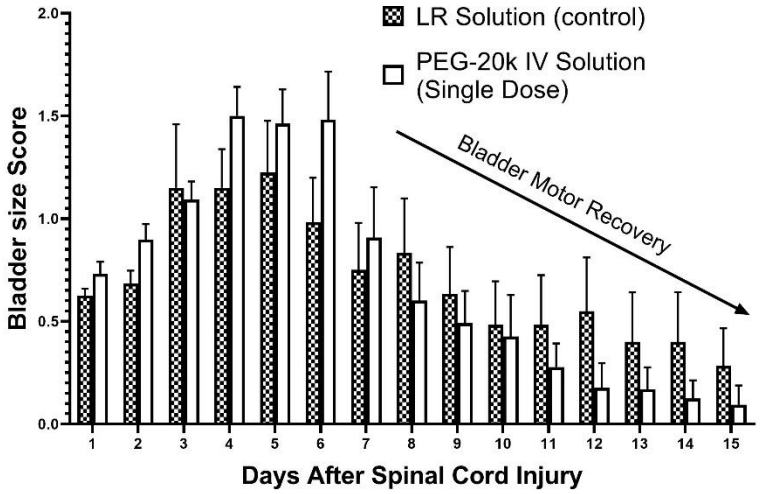
Score	**Interpretation**
0	No observable movement
1	Slight movement at the hip or knee
2	Extensive movement of one joint, and/or slight movement of another joint. (ankle, foot, or hip)
3	Extensive movement of two joints (ankle, foot, or hip)
4	Slight movement of all three joints (ankle, foot, or hip)
5	Slight movement of two joints and extensive movement of a third joint (ankle, foot, or hip)
6	Extensive movement of two joints
7	Extensive movements of all 3 joints of hindlimb, but no weight support
8	Sweeping movements with no weight support, or plantar placement with no weight support
9	Plantar placement and weight support while in stance only, occasional weight supported steps
10	Fewer than 50% weight supported plantar steps without front-hind limb coordination
11	50-90% weight supported steps without front/hind limb coordination
12	50-90% weight supported steps with LESS THAN 50% front/hind limb coordination
13	50-90% weight supported steps WITH 50-94% front/hind limb coordination
14	95-100% weight supported steps with 95-100% front-hind limb coordination AND Paw position is externally or internally rotated when it makes INITIAL CONTACT + Paw Position is externally or internally rotated when it is lifted off surface at the end of stance OR 50-90% plantar stepping, more than 95% coordination, and less than 50% dorsal stepping
15	95-100% plantar stepping with 95-100% coordination, but NO TOE clearance or fewer than 50% toe clearance with steps
16	95-100% plantar stepping with 95-100% coordination, and 51-94% toe clearance. Paw may be rotated at lift off, but must be flat/parallel during INITIAL CONTACT
17	95-100% plantar stepping with 95-100% coordination, and 51-94% toe clearance. Paw must be parallel/flat in plantar placement at LIFT OFF and at INITIAL CONTACT
18	95-100% plantar stepping with 95-100% coordination, and 95-100% toe clearance with steps.
19	95-100% plantar stepping with 95-100% coordination, and 95-100% toe clearance with steps. Paw must be parallel/flat in plantar placement at LIFT OFF and at INITIAL CONTACT. Tail is DOWN part or all the time.
20	95-100% plantar stepping with 95-100% coordination, and 95-100% toe clearance with steps. Paw must be parallel/flat in plantar placement at LIFT OFF and at INITIAL CONTACT. Tail is UP 95-100% of the time. Rat has TRUNK instability.
21	95-100% plantar stepping with 95-100% coordination, and 95-100% toe clearance with steps. Paw must be parallel/flat in plantar placement at LIFT OFF and at INITIAL CONTACT. Tail is UP 95-100% of the time. Rat has TRUNK STABILITY.

We have previously optimized the specific polymer mix that crosses the blood spinal cord barrier and that appears to promote maximal water transfer out of injured cord tissue after SCI. We also determined that one dose, while being effective in the cord, was not as effective as multiple doses after injury. However, additional smaller doses after a dose of 6.8 ml/kg, IV in injured rats would often exacerbate post-injury gastroparesis. This would often prevent the rats from drinking water and eating properly and cause breathing issues and sometimes aspiration pneumonia. This happens only in injured rats, only with repeated dosing, and it is reversible after 3-4 days. It has recently been determined that it DOES NOT happen in pigs, either normal or shocked. This level of gastroparesis would not prove to be a problem in clinical treatment of patients with SCI, should it occur in humans, since they would be fed by a g tube or a J tube until it resolves. Many rats with this condition survive the 3-4 day event on their own and these rats performed better during their recovery period since all of them showed no signs of neurogenic bladder, compared to the LR controls that always displayed neurogenic bladder. The loss of neurogenic bladder in the PEG-20k treated group could be a foreshadowing of better outcomes in the spinal cord motor function tests. We decided to test the effects of a single dose of PEG-20k IV solution after SCI in rats and compare this group to the vehicle control group that received just LR solution at the same volume and rate, even though we know the dose is not probably optimal. We have developed a surgical treatment of the rats for future testing of multiple doses that mitigates the gastroparesis through the completion of a sleeve gastrectomy in all animals. This removes the major volume of the stomach. This will be the focus of our efforts in the next months. This maneuver is an experimental method that allows the experiment to be completed at the higher dosages but would not be an issue in patients since it may not occur in humans and if it did occur, it would be treated medically for the short period of paresis. But the results of 16 rats (half given PEG-20k and half given LR solution) is shown after extensive testing of spinal cord motor function using the BBB assay using the single dose. The results are shown in **figure 17**.



Clearly, the data indicate that there are no differences in spinal cord motor function in the two groups. But since the spinal cord demonstrates secondary swelling after the 24-hour treatment period with the single dose of PEG, this was not surprising. Some additional dosing probably is required for an optimal effect, based on the kinetics of cord swelling and the pharmacokinetics of PEG-20k.

Figure 18 shows the bladder scores in the two groups of rats that were given the one single dose of PEG-20k or vehicle after SCI.

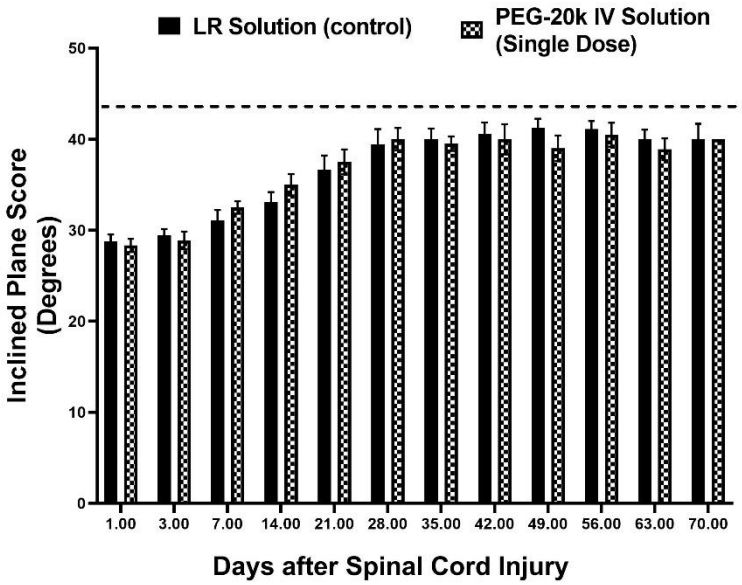


No effect of neurogenic bladder was seen with PEG-20k given as one dose. However, the return of bladder function was significantly faster with the single PEG-20k dose, compared to the LR controls. A second dose of PEG-20k given after the first completely abolished neurogenic bladder development in the few rats that were able to be tested with the gastroparesis.

In conclusion, we have some definitive results that shows that one single dose of PEG-20k IV solution given immediately after SCI is not enough to cause a significant improvement in spinal cord motor function and did not affect neurogenic bladder, but quickened the return to bladder function

Inclined Plane test: We have previously optimized the specific polymer mix that crosses the blood spinal cord barrier and that appears to promote maximal water transfer out of injured cord tissue after SCI. We also determined that one dose, while being effective in the cord, was not as effective as multiple doses after injury. However, additional smaller doses after a dose of 6.8 ml/kg, IV in injured rats would often exacerbate post-injury gastroparesis. This would often prevent the rats from drinking water and eating

properly and cause breathing issues and sometimes aspiration pneumonia. This happens only in injured rats, only with repeated dosing, and it is reversible after 3-4 days. It has recently been determined that it DOES NOT happen in pigs, either normal or shocked. This level of gastroparesis would not prove to be a problem in clinical treatment of patients with SCI, should it occur in humans, since they would be fed by a g tube or a J tube until it resolves. Many rats with this condition survive the 3-4 day event on their own and these rats performed better during their recovery period since all of them showed no signs of neurogenic bladder, compared to the LR controls that always displayed neurogenic bladder. The loss of neurogenic bladder in the PEG-20k treated group could be a foreshadowing of better outcomes in the spinal cord motor function tests. We decided to test the effects of a single dose of PEG-20k IV solution after SCI in rats and compare this group to the vehicle control group that received just LR solution at the same volume and rate. We have developed a surgical treatment of the rats for future testing of multiple doses that mitigates the gastroparesis through the completion of a sleeve gastrectomy in all animals. This removes the major volume of the stomach. This will be the focus of our efforts in the next months. This maneuver is an experimental method that allows the experiment to be completed at the higher dosages but would not be an issue in patients since it may not occur in humans and if it did occur, it would be treated medically for the short period of paresis. But the results of 20 rats (half given PEG-20k and half given LR solution) is shown after extensive testing of spinal cord motor function using the inclined plane test using the single dose. The results are shown in **Figure 19.**



Clearly, the data indicate that there are no differences in spinal cord motor function in the two groups. But since the spinal cord demonstrates secondary swelling after the 24-hour treatment period with the single dose of PEG, this was not surprising. Some additional dosing probably is required for an optimal effect, based on the kinetics of cord swelling and the pharmacokinetics of PEG-20k.

In conclusion, we have some definitive results that shows that one single dose of PEG-20k IV solution given immediately after SCI is not enough to cause a significant improvement in spinal cord motor function.

Overall Conclusions: Resuscitation of small and large animals with PEG-20k IV solution is remarkably effective because the inert polymer acts as an osmotic agent to rapidly pull metabolic water out of cells and tissues to open up compressed capillaries and restore microvascular perfusion and oxygenation. This reduced swelling occurs in traumatized spinal cord. The solutiuon developed for spinal cord injury has some smaller PEG polymers since getting some of this material to move across the cord capillaries requires a smaller molecular radius due to the tighter and less permeable capillary spaces. However, long term longitudinal studies lasting 2 months indicate that return of spinal cord function does not change in rodents immediately resuscitated with these polymer solutions, compared to the vehicle controls. We do see a significant faster return to normal bladder function after SCI with PEG polymers. There is also less glial inflammatory activation with PEG. Since we demonstrated that the smaller polymers are cleared more quickly, we tried increasing our effectiveness by increasing dosing of the rats from a single dose (which is highly effective in circulatory shock) to 2-3 doses. However, this caused a gastroparesis that left the animals in poor shape so we were never able to effectively test the multiple dose hypothesis. It is interesting that multiple doses of PEG-20k IV solution in swine do not have these effects on gastric paralysis. Probably the rodent GI system is different enough to see the adverse side effect. Finally, we believe the effectiveness of PEG on spinal cord function would be greatly amplified in a Polytrauma model where cardiovascular shock is also present with SCI, which would commonly occur on the battlefield space. However, we developed the Polytrauma model but was unable to test the drug due to countless delays and lost productivity due to COVID and secondary COVID effects on university support personnel. While these effects were mostly negative, we feel that the treatment can work and be optimized in patients and want to move to a non-rodent model such as swine SCI to fully test the hypothesis.

4. **Impact:** Short-term benefits of these polymer-based resuscitation solutions in cardiovascular performance and neuroprotection will lead to long-term functional benefits in injured warfighters. Short term immediate tissue stabilization of spinal cord injuries in the field prevents the long term (days to weeks) sequelae of compounded tissue injury from inflammation and demyelination. A higher return of function for patient suffering from moderate to severe spinal cord injuries on the field should be realized longer term. The long term emotional, psychological, and physical defects unmasked by sudden paralysis could be reduced with superior treatment and stabilization of spinal cord tissue on the field immediately following trauma. Return to work provides emotional and monetary security for the patient and unit efficiency is improved for the Army by having highly trained injured soldiers return to the unit with full pre-injury motor function.

5. **Changes/Problems:** There were two problems encountered in this study: One was the COVID crisis and the effects this had on productivity due to lab shut downs and shortages of personnel, mostly in the animal facilities. The second was in the apparent physiology of the ani mal model. Rodents, with a different GI system, seem to be sensitive to more frequent dosing of PEG-20k resuscitation n solutions. Specifically, rodents develop a gastroparesis that threatened their life after 2 or more doses of PEG polymer solutions. This limited our ability to extend the osmotic effects of PEG in the spinal cord tissue to 48 hours, which is the time we found they swell after injury. Similar dosing schedules in swine, which have a GI system similar to humans, does not cause gastroparesis. Even if it did in patients, it would be easily manageable because it is reversible with time and the stomach can be decompressed with placement of a G-tube during treatment. This can't be done in oiur rodents so we were forced to limit the total dose of PEG and the time of treatment. As this project moves forward, we will seek to work with swine models to avert this problem.

6. **Products:** Two manuscripts for publication are being written and finalized for submission for publication.

7. **Participants & Other Collaborating Organizations:** The following personnel have participated at some time during this project.

Nancy Lee, BS
Research Technician
6 person months worked (50%)
Ms Lee is an animal technician performing the injuries and tests on the rodents

Caitlin Archambault, LVT
Lab Manager
3 person months (25%)
Caitlin is the lab manager for the project and the veterinary support technician. She oversees all of the details of the project and provides veterinary support for the animals. She also performs lab technical duties such as assays and tests.

Dr. Ru Li
Research Scientist
2.4 person months (20%)
Dr. Li is a staff scientist with expertise in molecular biology and imaging. He helps with immunohistochemical analysis of tisse specimens and conducts western blot, PCR, and ELISA analysis on spinal cord tissues recovered from the studies. He is running baseline assays now.

Dr. Martin Mangino
Principal Investigator, Professor of Surgery, and Associate Chair of Surgery
3.0 person months (25%)
Dr Mangino is the PI who oversees all scientific, personnel, and administrative roles of the project. He directs the team in the project objectives, analyzes data, provides resources, and conducts all of the administrative duties needed to run a large invvestigation (compliance, reports, publications, etc.).

Dr. Jad Khoraki
Post-doctoral Fellow
4 person months (33%) effort-Replacing Dr Rihane who is leaving in July.
Jad is a post-doc in Dr Mangino's lab and has considerable expertise in shock, trauma, and the many models of hemorrhagic shock used in the lab. He will begin assisting witjh the polytrauma arm of the study by inducing hemorrhagic shock in rats befor they receive SCI. He just started working with the SCI team.

Dr. Anna Xu
General Surgery research resident
4 person months (33% effort). Dr Xu is working in Dr Mangino's lab on this project. She has completed 2 years of a general surgery residency program and has now chosen to conduct the next 2 years working on trauma projects in Dr Mangino's lab. She has a neuroscience background and is supervising many of the daily details of the project.

Dr. Kirsty Dixon
Assistant Professor of Surgery (Co-I)
3.6 person months worked (30%)
Dr. Dixon is Co-I and is the neurotrauma expert on our team, overseeing the spinal cord injury and histological / neorbehavioural outcomes evaluations

Heather Reichstetter, LVT
Lab Manager
3 person months (25%)

Mrs. Reichstetter is the lab manager for the project and the veterinary support technician. She oversees all of the details of the project and provides veterinary support for the animals. She also performs lab technical duties such as assays and tests.

Dr Naima Rihane
Post-doctoral Fellow
6 person months (50%)
Dr Rihane is a postdoc with previous experience in neuroinflammation and neuroscience research. She will be conducting most of the technical studies on the project and analyzing data and organizing the day to day operations. She reports to Dr Mangino and works with Dr Dixon.

Jerry Maitland, BS
Research Technician
6 person months (50%)-Just started 3/19
Jerry is a recent VCU graduate with a degree in biology and prior experience working in a neurotrauma lab in the Pharmacology Department. He is already familiar with many of the behavioral and motor function tests. Jerry will work with Drs Dixon and Mangino on this project.

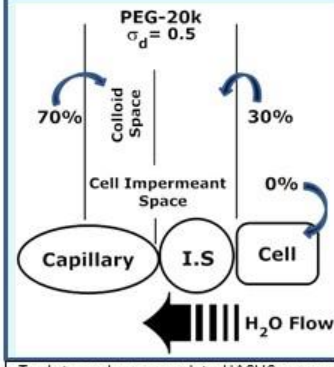
8. **Special Reporting Requirements:**

Treatment of Spinal Cord Ischemia with Cell Impermeant-Based Resuscitation
SC160218
W81XWH-17-1-0602

PI: Martin J Mangino, PhD **Org:** Virginia Commonwealth University **Award Amount:** $1,620,499

Study/Product Aim(s)
1.) To determine the optimal molecular weight PEG polymer in spinal cord microcirculation for use in spinal cord injury (SCI) models
2.)To determine the effects of spinal cord-optimized PEG polymers in resuscitation solutions for treatment of SCI and complex trauma

Optimized polyethylene glycol (PEG) polymer sizes will be determined that provide an osmotic reflection coefficient in the spinal tissue of 0.5. Then, these polymers will be tested in a low volume resuscitation (LVR) crystalloid solution for the ability to prevent spinal cord injury in rat models of spinal cord injury with and without complex injury involving hemorrhagic shock. The goal is to develop a stable LVR crystalloid for pre-hospital use that both reduces spinal cord swelling injury and provides increased tolerance to the low volume state in lethal hemorrhagic shock. This increases safe transport times.



Basic mechanism involves finding the optimal size PEG polymer to provide a double osmotic gradient in the spinal cord microcirculation. When used as a resuscitation solution, this will rapidly pull metabolic water from the injured cord tissue, thus preventing injury by decompression and enhanced O2 transfer. The approach is used early in the field enhancing transport outcomes

To date we have completed IACUC approval, trained personnel, and successfully cannulated the spinal canal of rodents to be able to begin measuring polymer osmotic reflection coefficients in spinal microcirculation. Injury controls have started too.

Timeline and Cost

Activities CY	18	19	20	
Polymer optimization for solution				
Test in spinal cord injury (SCI)				
Test in Hemorrhagic shock (HS)				
Test in both SCI and HS (polytrauma model)				
Estimated Budget ($K)	**$529**	**$540**	**$551**	

Updated: (Dec 10, 2021)

Goals/Milestones
CY18 Goal – PEG Polymer size optimization
☑ Polymer chain length (mass) and branching effects on osmotic reflection coefficient in spinal tissue to customize a CNS tissue specific LVR solution to prevent cord swelling
CY19 Goals – Test optimized polymer solutions in rat models
☐ Test in spinal cord injury (SCI) models
☐ Test in Hemorrhagic shock (HS) with PEG-20k (optimized for HS)
CY20 Goal – Finish testing in animals
☐ Test new solution in SCI and HS models combined (polytrauma)
☐ Lab analytical work: Tissue processing (PCR, WB, IHC)

Comments/Challenges/Issues/Concerns: None yet noted.

Budget Expenditure to Date
Projected Expenditure:$1,620,000 Total Costs
Actual Expenditure: :$1,620,000 Total Costs :

9. **Appendices:** None