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TITLE: A Novel Advanced Resuscitation Fluid for Traumatic Brain Injury with Hemorrhagic Shock

PRINCIPAL INVESTIGATOR: Denis E. Bragin

RECIPIENT: University of New Mexico Health Sciences Center
1 University of New Mexico,
MSC 10 6515, Neurosurgery,
Albuquerque, NM, 87131, USA

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6. AUTHOR(S) Denis E Bragin

E-Mail: dbragin@salud.unm.edu

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University of New Mexico Health Sciences Center
1 University of New Mexico, MSC 10 6515, Neurosurgery, Albuquerque, NM, 87131, USA

McGowan Institute for Regenerative Medicine, University of Pittsburgh
450 Technology Drive, Suite 300
Pittsburgh, Pennsylvania
15219, USA

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13. SUPPLEMENTARY NOTES

14. ABSTRACT
Traumatic brain injury (TBI) is frequently accompanied by hemorrhagic shock (HS) which significantly worsens neurologic outcome, and increases mortality. Current resuscitation fluids (RF) for volume expansion after TBI with HS do not adequately ameliorate impaired microvascular cerebral blood flow (mvCBF). We suggested the addition of drag reducing polymers (DRP) to resuscitation fluid (DR-RF) for TBI with HS which will reduce the severity of brain injury, increase survival rate, improve neurologic recovery and will reduce the volume of resuscitation fluid required to prevent the transition to an irreversible stage and death or functional impairment of the brain. The purpose for the proposed research is to apply DRP as an additive to resuscitation fluids after TBI with HS, to determine which mechanisms are affected by DRP in the acute and late recovery phases and to define most effective parameters for application. During reported period we showed that colloid, hypertonic and colloid-based DRP-RF significantly improves cerebral regional and microvascular circulation and tissue oxygenation impaired by TBI/HS. Effect lasts at least 6 hours. Colloid-based DRP-RF was more effective than crystalloid and hypertonic—based DRP-RF tested. We have also done evaluation of TBI/HS-induced metabolic stress of mitochondria, hypoxia, neuronal survival and microthrombosis and beneficial effects of DRP-RF-vs. RF. Sub-Contractor performed experiments on DRP characterization and storage and drag reduction test circuit development. The results were presented on 4 conferences, one manuscript published, one accepted and two are in preparation.

15. SUBJECT TERMS
Traumatic Brain Injury with Hemorrhagic Shock, Resuscitation Fluid, Drag Reducing Polymers, Animal Models, Cerebral Microcirculation, Neuroprotection.

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1. **INTRODUCTION:** Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.

Traumatic brain injury (TBI) is frequently accompanied by hemorrhagic shock (HS) which significantly worsens neurologic outcome, and increases mortality. Current resuscitation fluids (RF) for volume expansion after TBI with HS do not adequately ameliorate impaired microvascular cerebral blood flow (mvCBF). In our previous studies in a rat TBI model, we have shown that nanomolar concentrations of intravascular blood soluble drag reducing polymers (DRP) significantly enhanced microvascular perfusion and tissue oxygenation in peri-contusional areas thereby protecting neurons. We hypothesized the addition of DRP to resuscitation fluid (DR-RF) for TBI with HS reduces the severity of injury, increases survival rate, improves neurologic recovery and will reduce the volume of resuscitation fluid required to prevent the transition to an irreversible stage and death or functional impairment of the brain. The purpose for the proposed research is to apply DRP as an additive to resuscitation fluids after TBI with HS, to determine which mechanisms are affected by DRP in the acute and late recovery phases and to define most effective parameters for application. The proposal fits well with all 3 Focus Areas of PFCRA: 1) Understand the clinical implications of PFC and pDCR, including "physiological parameters requiring intervention to reduce morbidity and mortality during the acute treatment of TBI and mitigation of the pathophysiology of prolonged hypotension"; 2) Develop next-generation resuscitation methods for PFC and pDCR, including "novel or improved methods for resuscitation and stabilization of TBI/HS, with or without other concomitant injuries; and 3) Develop enhanced treatment of injuries during PFC and pDCR, including "TBI treatments to reduce tissue loss, ischemia, secondary injury mortality and improve outcomes.

2. **KEYWORDS:** Provide a brief list of keywords (limit to 20 words).

Traumatic Brain Injury with Hemorrhagic Shock, Resuscitation Fluid, Drag Reducing Polymers, Hemorheological Approach, Animal Models, Cerebral Microcirculation, Neuroprotection

3. **ACCOMPLISHMENTS:** The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction.

What were the major goals of the project?
List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

Specific Aim 1: Elucidate the major mechanisms and beneficial effects of DRP resuscitation fluid (DRP-RF) infused in animals at the "pre-hospital" acute phase up to 6 hours after traumatic brain injury with hemorrhagic shock (TBI/HS) compared to crystalloid or colloid fluids in controls and contrasted to HS or TBI only using sham as a control

Major Task 1: Evaluate effectiveness of DRP-RF in improvement of microvascular cerebral blood flow and preventing blood brain barrier degradation

Subtask 1. Laser speckle contrast imaging of changes in regional cerebral blood flow (rCBF) – 1-24 months – 100%

Subtask 2. Two-photon microscopy of changes in microvascular and quantitation of capillary density – 1-24 months – 100%

Major Task 2: Evaluate efficacy of DRP-RF in improving oxygen delivery to brain tissue after TBI/HS

Subtask 1. Multispectral optical intrinsic signal imaging of regional changes of oxy- and deoxy-hemoglobin concentration 1-24 months – 100%

Subtask 2. Two-photon microscopy of change in brain tissue oxygenation via nicotinamide adenine dinucleotide (NADH) fluorescence imaging 1-24 months – 100%

Major Task 3: Evaluate the effect of DRP-RF on oxidative stress and survival of neurons after TBI/HS

Subtask 1. 2PLSM imaging of i.v. injected hydroethidine to visualize superoxide – a major component of oxidative stress density – 1-24 months – 100%

Subtask 2. 2PLSM imaging of i.v. injected propidium iodide for visualization of dying neurons – 1-24 months – 100%

Major Task 4: Test the effect of DRP-RF in TBI/HS by physiological monitoring

Subtask 1. Monitoring of changes in physiological parameters including intracranial pressure, mean arterial pressure, pulse rate, cortical Doppler flow, and analysis of blood gases, electrolytes, hemoglobin, glucose/lactate, pH and coagulation – 1-24 months – 95%

Subtask 2. Evaluation of quantitative changes in cerebrovascular autoregulation with DRP-RF compared to crystalloid and colloid fluid resuscitation after TBI/HS– 1-24 months – 95%.

Sub Aim 1a: Optimization of the DRP-RF preparation process for combat casualty use

Major Task 1: Optimization of a process of preparation, sterilization, and storage conditions for creation of the concentrated DRP-RF which will be usable within a few minutes – 1-30 months – 95%

Specific Aim 2: Compare the beneficial effects of DRP-RF on long-term recovery and neurologic outcomes compared to crystalloid and colloid fluid treatments for up to 4 weeks after TBI/HS.

Major Tasks 1: Evaluate behavioral outcomes

Subtask 1. Sensory and coordination motor deficits (Adhesive removal, Rotarod, Catwalk) – 20-32 months – 30%

Subtask 2. Cognitive deficits (Passive avoidance - learning, Y- maze working and Novel Object - recognition memory) – 20-32 months – 35%.

Major Tasks 2: Quantitative Magnetic Resonance Imaging voxel-wise evaluation of DRP-RF effect on recovery after TBI/HS

Subtask 1. Digital contrast enhanced (DCE) MRI for quantitative blood brain barrier evaluation with voxel-wise post processing – 20-32 months – 25%

Subtask 2. Quantitative DTI for apparent diffusion coefficient (ADC), quantitation of edema and contusion volume – 20-32 months – 25%.

Subtask 3. Quantitative measurement of CBF by pulsatile arterial spin labeling and voxel-wise post processing and frequency histogram analysis– 20-32 months – 25%.

Major Tasks 3: Histochemical assessment at sacrifice time-point comparing DRP-RF and crystalloid and colloid fluid resuscitation after TBI/HS and long term recovery

Subtask 1. Neuronal death evaluation (necrosis, apoptosis, H&E) – 20-32 months – 25%
Subtask 2. Capillary density evaluation by alkaline phosphatase staining – 20-32 months – 25%.
Subtask 3. Inflammation evaluations (microglia and astrocytes activation) – 20-32 months – 25%

Major Tasks 4: Data analysis and interpretation for Specific Aim 2 – 24-36 months – 35%

What was accomplished under these goals?
For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

For the reported period, we have completed Specific Aim 1 – Elucidation of the major mechanisms and beneficial effects of DRP resuscitation fluid (DRP-RF) infused in animals at the "pre-hospital" acute phase up to 6 hours after traumatic brain injury with hemorrhagic shock (TBI/HS) compared to crystalloid, colloid or hypertonic fluids in controls and contrasted to HS or TBI only using sham as a control.

This included four Major Tasks where we evaluated: Improvement of microvascular cerebral blood flow and preventing blood brain barrier degradation (1); Improving oxygen delivery to brain tissue (2); the effect of DRP-RF on oxidative stress and survival of neurons (3); and physiological monitoring (4).

On Fig. 1 is presented general experimental protocol which, with variations, was used for the reporting period. TBI was induced after baseline in-vivo 2-photon laser scanning microscopy (2PLSM) and followed by a 1-h hemorrhagic phase (Battlefield), where blood was slowly withdrawn through the femoral vein to reduce mean arterial pressure (MAP) to 40 mmHg. In the following 1-h pre-hospital care phase (Transportation, PFC), resuscitation fluids (LR-RF or DRP-RF) were slowly infused i.v. to raise MAP to ~55 mmHg and CBF to ~65% of baseline. In a subsequent 3-h definitive hospital care phase, shed blood was re-infused to a MAP of 70 mmHg and CBF of ~75% of baseline. *In vivo* 2PLSM or LSCM were done throughout the study over the parietal cortex of the rat brain. Brain and rectal temperatures were monitored and maintained at 38 ± 0.5°C. Arterial blood gases, electrolytes, hematocrit and pH were measured hourly (epoc Blood Analysis System, Alere Inc., Waltham, MA, USA).

Experiments on rats with crystalloid RF vs crystalloid RF+DRP, colloid RF vs colloid RF+DRP and hypertonic RF vs hypertonic RF+DRP were performed. Laser speckle contrast imaging and two-photon microscopy showed that addition of DRP to RF significantly improves cerebral regional and microvascular circulation and tissue oxygenation, reduced by TBI/HS (Fig. 2). The effect persisted during the whole monitored period (6 hours). The results are presented in the published manuscript attached. Colloid-based DRP-RF was more effective than crystalloid, and hypertonic—based DRP-RF.

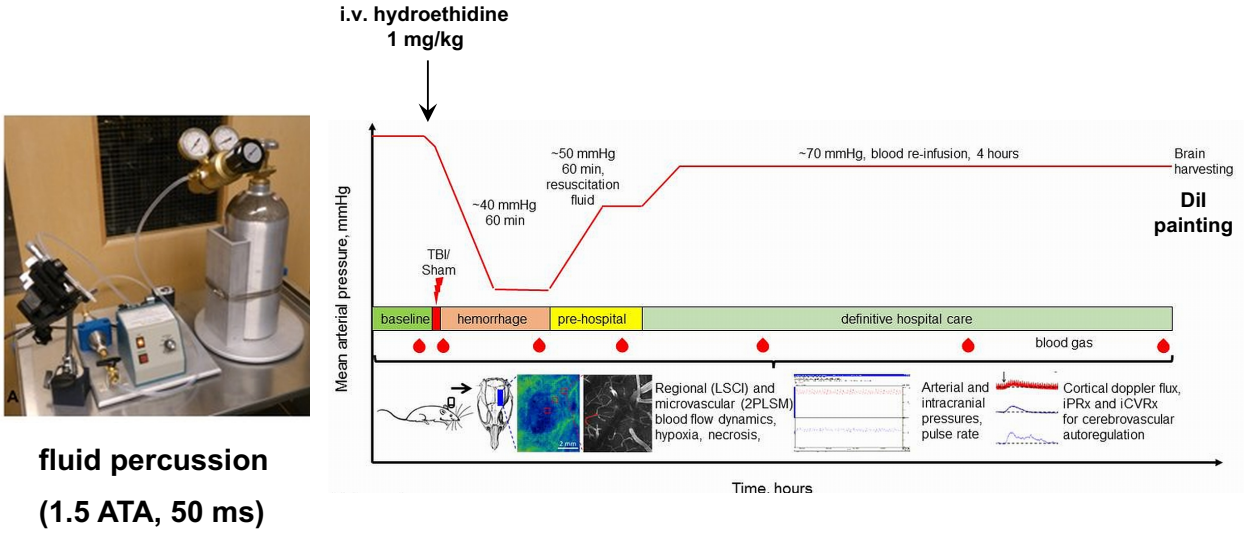


Fig. 1 Experimental Protocol

We have evaluated TBI/HS-induced metabolic stress of mitochondria that leads to excessive oxidative phosphorylation and the increased production of reactive oxygen species (ROS) such as superoxide. Using 2PLSM imaging of i.v. injected hydroethidine to visualize superoxide, we have shown that TBI/HS lead to oxidative stress which was less in a group resuscitated with DRP added fluid (Fig. 3). The results are presented in the accepted manuscript attached.

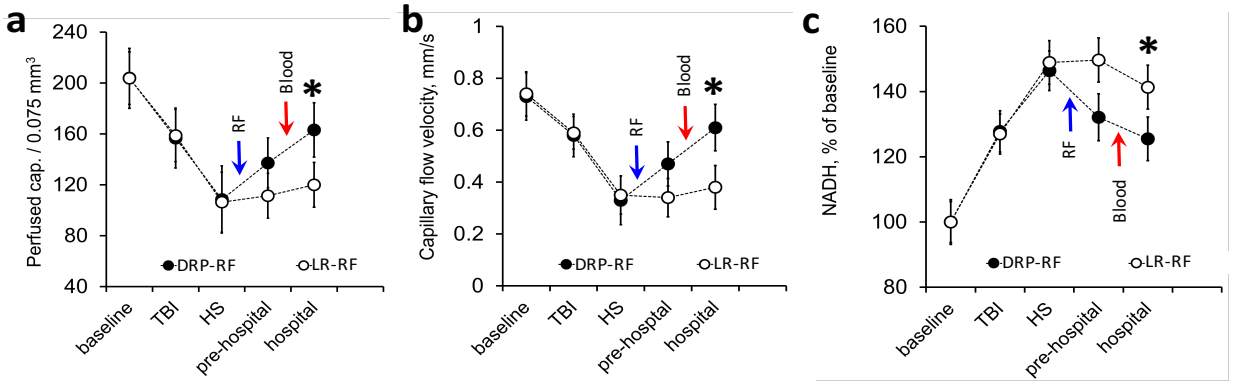


Fig. 2 Resuscitation with crystalloid based DRP-RF improves cerebral microvascular perfusion and tissue oxygenation impaired by TBI/HS, as shown by a) increased number of perfused capillaries; b) increased capillary flow velocity; c) increased tissue oxygenation (NADH decrease). Mean ± SEM, N=10 rats per group, *P < 0.05 from the LR-RF group.

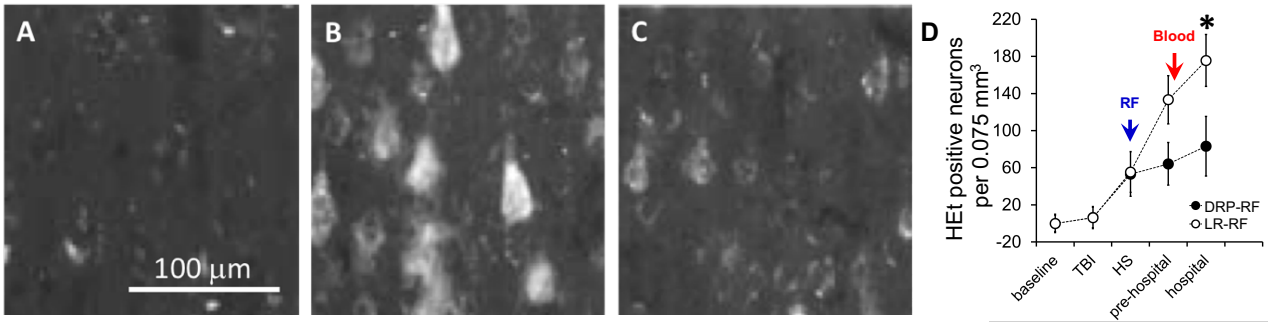


Fig. 3 Resuscitation with DRP-RF reduces superoxide production in cortical neurons after TBI with HS: a) Representative image of a rat cortex at baseline without ET positive neurons; b) Neurons with diffuse cytosolic ET fluorescence in a rat cortex from LR-RF group by the end of experiment; c) and from DRP-RF group; The dynamics of the increase in ET positive cortical neurons. Mean ± SEM, N=10 rats per group, *P < 0.05 from the LR-RF group.

Using DiI vascular painting technique, we have evaluated microvascular changes in extracted brain and found massive microthrombosis in both, contralateral and ipsilateral to trauma hemispheres, that was less in DRP-RF groups. In the injured hemisphere in DRP-RF, microvascular density was higher than in LR-RF (% vessel/total area*100 was 4.9 ± 0.4 vs. 3.1 ± 0.3, respectively, p<0.05) as oppose 6.8 ± 0.4 to in Sham rat. In contralateral to the injury hemisphere, microvascular density was also reduced (% vessel/total area*100 was 6.1 ± 0.5 vs 5.2 ± 0.5, in DRP-RF, vs. LR-RF, respectively, p<0.09) (Fig. 4). The results are presented in the accepted manuscript attached.

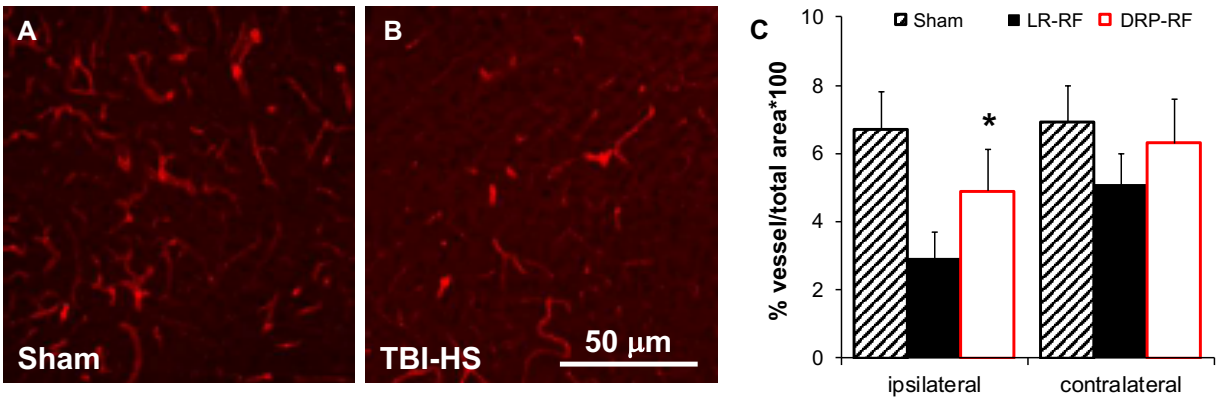


Fig. 4 Resuscitation with DRP-RF reduces microthrombosis in both hemispheres after TBI with HS as shown by post-mortem DiI vascular painting. a) Cortical microvascular network in Sham mouse brain; and b) after TBI with HS; c) Graph showing reduced cortical microvasculature in LR-RF group and better-preserved microvasculature in DRP-RF group in both, traumatized and contralateral hemispheres. Mean ± SEM, N=10 rats per group, *P < 0.05 from the LR-RF group.

To evaluate *neuronal survival*, 200 µL of a propidium iodide (PI)/saline, which labels only necrotized cells with damaged membrane, was injected intravenously during surgical preparation. CBF and tissue oxygenation reduction after TBI/HS caused progressive necrosis of neurons. DRP-RF reduced progression of necrosis of neurons while standard RF (LR) did not decrease dynamic of necrosis. Fig. 5). The results are presented in the published manuscript attached.

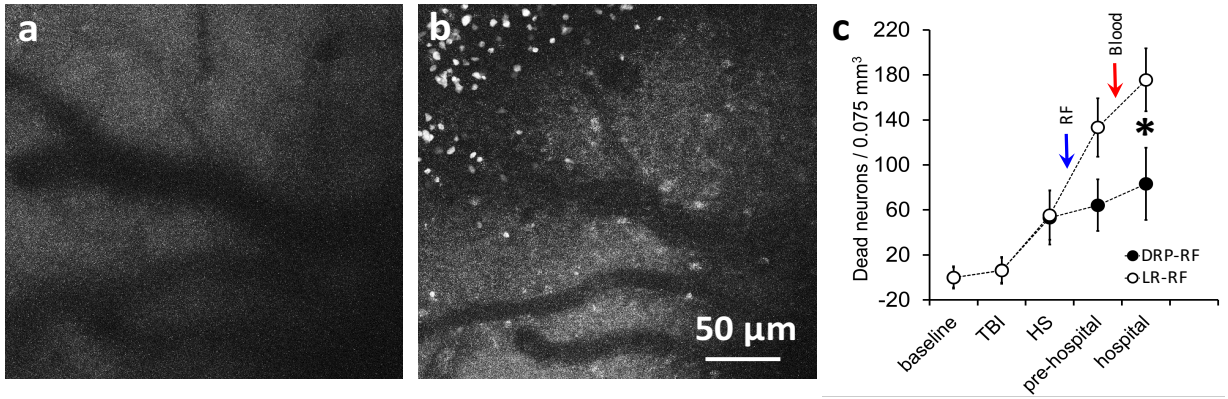


Fig. 5 Resuscitation with DRP-RF is neuroprotective: a) 2PLSM image of a rat cortex at baseline without dead neurons; b) Propidium Iodide stains neurons with damaged membranes reflecting necrosis of neurons after TBI/HS; c) DRP-RF protects neurons from necrosis (*=P<0.05). Mean ± SEM, N=10 rats per group, *P < 0.05 from the LR-RF group.

The anticipated parts of Major Tasks 1-4 of the Specific Aim 1, and thus, this period goals, were completed

We have been working on the Specific Aim 2: (Compare the beneficial effects of DRP-RF on long-term recovery and neurologic outcomes compared to crystalloid, colloid and hypertonic fluid treatments for up to 4 weeks after TBI/HS.

This included four Major Tasks evaluated: Evaluate behavioral outcomes (1); Quantitative Magnetic Resonance Imaging voxel-wise evaluation of DRP-RF effect on recovery after TBI/HS (2); Histochemical assessment at sacrifice time-point comparing DRP-RF and crystalloid and colloid fluid resuscitation after TBI/HS and long-term recovery (3); Data analysis and interpretation for Specific Aim 2 (4).

In particular we have shown the dynamic of CBF during 4 weeks after TBI/HS and the effect of DRP enforcement of resuscitation fluids using MRI. Behavioral testing revealed improvement in neurologic outcome. Some of the results are presented below:

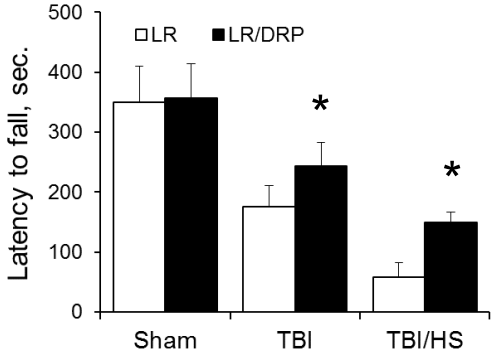


Fig. 6 DRP improves sensory-motor function as the latency to fall, evaluated by the rotarod test, is significantly longer in DRP groups than in controls (LR) at 1 week after the insult.

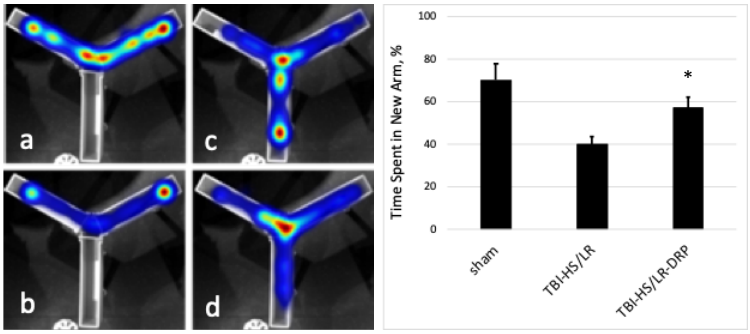


Fig. 7 improves spatial working memory as measured by Y-maze: a) Intact rat actively investigate the open two hand of the Y-maze; b) traumatized rat is not active; c) after opening of the third hand of the Y-maze, intact rat actively investigates the new hand of the maze; d) due to deficiency in the spatial working memory, traumatized rat evenly investigates all hand of the maze and is inactive in general. The graph on the right shows better preserved/recovered spatial memory in DRP group.

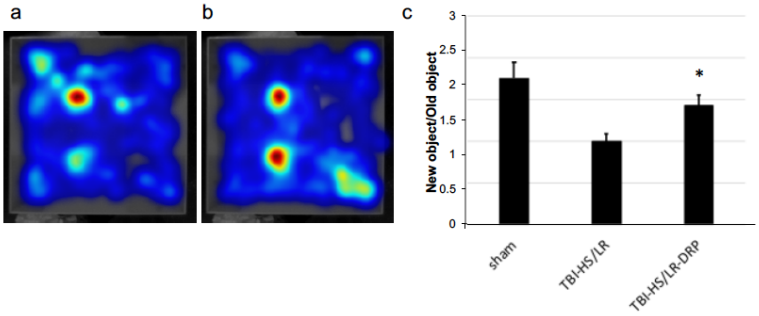


Fig. 8 DRP improves cognitive memory as measured by Novel object recognition: Short-term cognitive memory (a) The color heat map of the moving intact mouse that spends more time exploring the novel object (b) The color heat map of the mice after TBI/HS, showing memory impairment, since the mouse equally examines both objects. (c) The graph showing better preserved/recovered cognitive memory in DRP group, * $p < 0.05$.

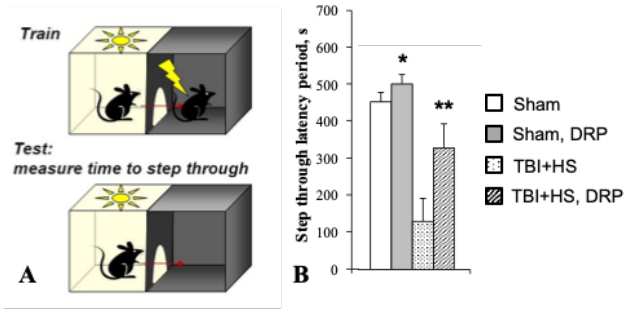


Fig. 9 DRP addition to Hetastarch improves learning and memory in rats at 1 week TBI and HS measured by Passive Avoidance test.

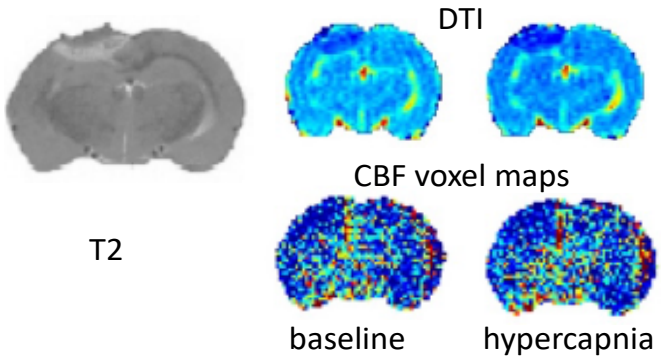


Fig. 10 Representative MRI images showing cerebral vascular reactivity (CVRx) changes after TBI/HS measured by MRI and hypercapnia test. Resuscitation with DRP better preserved CVRx.

The University of Pittsburgh sub-awardee near-completed Sub Aim 1a: Optimization of the DRP-RF preparation process for combat casualty use. This included one Major Task 1: Optimization of a process of preparation, sterilization, and storage conditions for creation of the concentrated DRP-RF which will be usable within a few minutes.

DRP characterization and storage:

Drag-reducing polymers (DRPs) such as polyethylene oxide (PEO) degrade when exposed stressors such as extreme shear. The degradation of these polymers leads to their reduced effectiveness through decreased molecular weight which may be measured via solution viscosity. In preparation for the use of DRPs in chronic/long-term pre-clinical studies and their eventual clinical use, the various degradation mechanisms which these polymers may become subject to during their production and storage must be studied in order to establish suitable stabilization techniques to prevent degradation. In particular, this project aims to study DRP degradation caused by various sterilization techniques, mechanical fragility tests, and exposure to light sources. PEO solutions (4M Molecular Weight, Sigma Aldrich, USA) at concentrations ranging between 250 ppm to 5000 ppm is prepared using the following solvents: water, normal saline, and ethanol. Sterilization via vacuum-driven and pressure-driven filtration systems is ongoing over a range of filtration rates. We began to use an ultraviolet light source (sun lamp or light therapy box) to test degradation of DRPs via light exposure for up to 40 hours and will be compared to samples stored in the absence of light. Finally, the mechanical fragility of the polymer solution is determined using a Brookfield rheometer and rocker bead tests similar to experiments also performed in our laboratory to determine the mechanical fragility of RBC suspensions and whole blood. The viscosity of all solutions is measured after each degradation technique is performed to determine percent viscosity loss due to degradation. Establishing sterilization procedures to maintain DRP effectiveness and identifying modes of degradation (i.e. light exposure or mechanical stress) would help to produce standard protocols for the use of DRPs in future pre-clinical and clinical studies.

In another set of experiments, polyethylene oxide (PEO) solutions (4M MW, Sigma Aldrich, USA) at concentrations of 1000 ppm and 500 ppm were created and their viscosity and elasticity were determined using the Vilastic-3 viscoelasticity analyzer (Vilastic Scientific, Inc., Austin, TX, USA) over shear rates from 20 to 500 s^{-1} (below). The Vilastic-3 was used in preference over the Brookfield rheometer which was used in previous storage testing because it was found that a higher

sample repeatability could be attainted, which will enable better depiction of changes in DRP due to storage degradation. Solutions of prepared mixtures were separate into aliquots and stored at 4.5ºC and -19ºC for preparation of our now ongoing storage degradation study using. Samples will be measured roughly every three weeks for DRP viscosity and elasticity to ensure sample viability. Our newest characterization and storage studies are observing the degradation of 500 and 1000 ppm PEO, as opposed to previous values of 1000 and 4000 ppm, to more closely imitate concentrations that PEO solutions could be stored at in clinical setting and compare that lower concentration to the already tested 1000 ppm. Future tests will focus on concentrations that are even lower than 500 ppm.

Progress:

Storage-induced degradation was performed previously on PEO solutions at concentrations of 1000 ppm and 4000 ppm in sterile saline. There was found to be an insignificant degree of viscosity loss over the storage course of 6 weeks when stored at -20ºC, however further testing of storage-induced degradation of lower concentration solutions may be needed as the current usage of these DRP solutions in-vivo requires injection of solution 50 ppm or less.

Vilastic-3: The Vilastic-3 determines both viscosity and elasticity with high precision. It does this by modulating sample flow in a sinusoidal manner within a small metal tube. As the machine continuously alters flow of the fluid, the pressure generated at the top of the tube is also measured. The magnitude and phase change differences between pressure and flow measurements are used to determine both viscosity and elasticity of the sample. Sample response over a wide range of shear rates is made possible by increasing the drive pressure and frequency. The viscosity of stored PEO solutions is important for this work as it directly correlates to the integrity of the polymer; low MW and degraded polymer will have lower viscous properties.

Preliminary light degradation tests were performed using PEO 2000 ppm and PEO 5000 ppm in both sterile water and sterile normal saline. DRP viscosity and elasticity were measured using Vilastic-3 viscoelasticity analyzer (Vilastic Scientific, Inc., Austin, TX, USA) over shear rates from 1 to 500 s^{-1} at room temperature (20ºC), Figure 1. Exposure to a commercial plant grow light (Feit Electric, BR30/GROW/LEDG2 LED) for 40 hours showed slight to no signs of PEO degradation, however exposure to sunlight as well as UV light exposure from within a biosafety cabinet caused significant decreases in both viscosity and elasticity, indicating degradation (Fig. 2). The lack of DRP viscosity degradation using the grow light is most likely due to the unique emission spectrum designed for plant growth which does not reach the region of ultraviolet light required to induce free radical polymer chain degradation. Additionally, mechanical degradation via shear stress within a Brookfield rotational viscometer was found to be able to degrade DRP, but the relatively small amount of shear stress applied (15-22 Dyne/cm^2) limited the test's ability to degrade polymer past 5% of its original viscosity. Other means of mechanical degradation such as rocker bead tests are currently being explored.

Finally, DRP sterilization via pressure driven syringe filter was performed using 0.22 um sterile filters. DRP solutions at 2000 ppm were driven at a rate of 2 mL/min through either polyvinyl difluoride (PVDF) or polyethersulfone (PES) membranes. The pressure drop across both types of membrane was approximately 600-700 mmHg. The viscosity of the DRP solutions was measured pre-sterilization and after passing through the filter a single time (Fig 3.). A slight loss of viscoelasticity was measured following this rapid filtration. Verification of sterilization (inoculation of DRP and its subsequent sterility following filtration) will be the next step of our study.

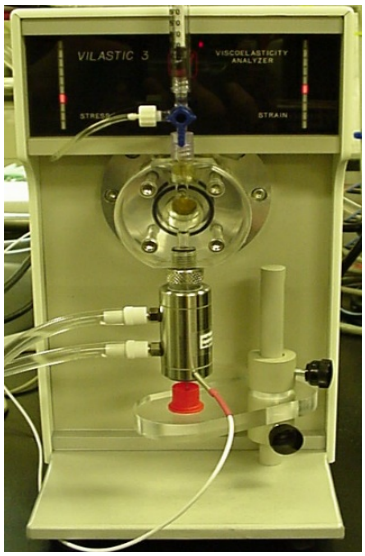


***Figure 1**. Vilastic Viscoelasticity Analyzer*

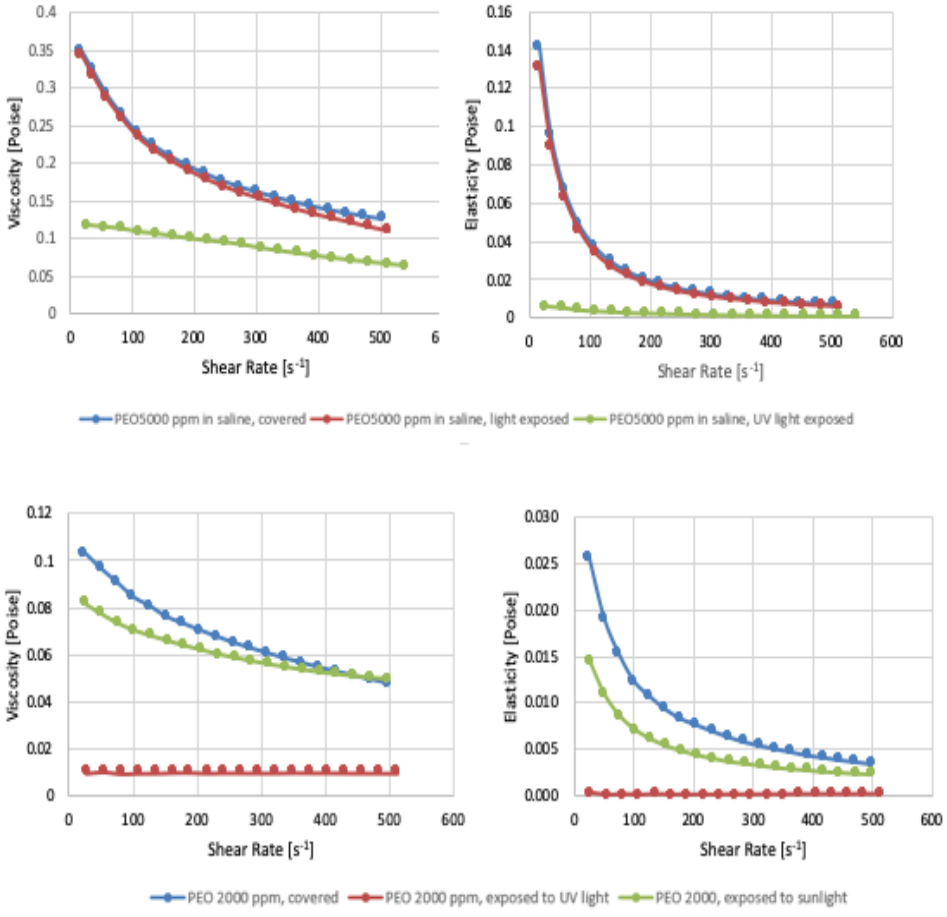


***Figure 2**. Viscosity and elasticity of PEO 5000 ppm solutions after exposure to no light (blue), grow light (red), and UV light (green) (TOP). Viscosity and elasticity of PEO 2000 ppm solutions after exposure to no light (blue), sunlight (green), and UV light (red) (BOTTOM).*

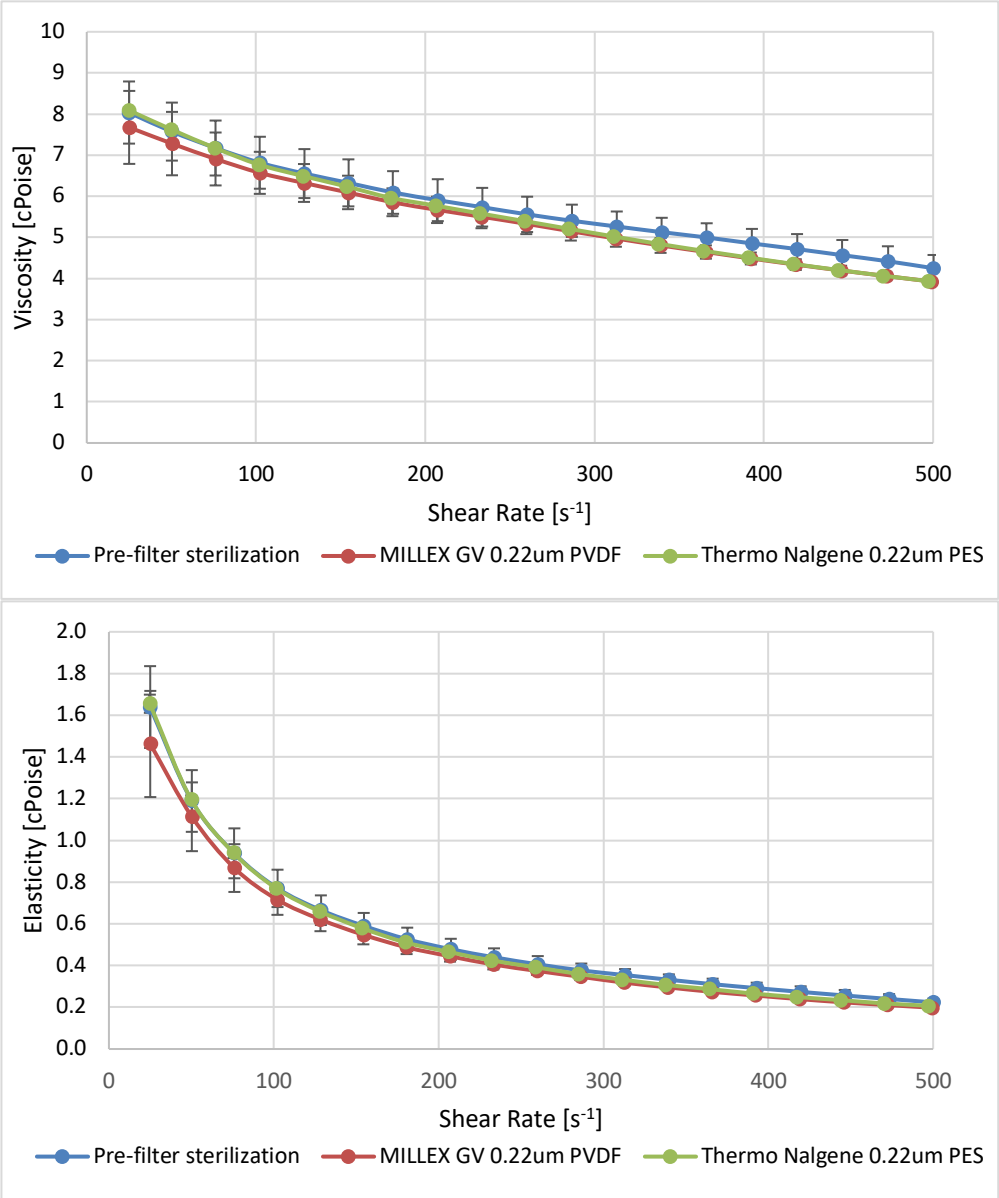


***Figure 3**. Viscosity (top) and elasticity (bottom) of 2000 ppm PEO solutions following sterilization of 0.22 filter membranes.*

Conclusions: The 1000 ppm PEO showed greater variation between the different days of the experiment. On the other hand, the average viscosity for 500 ppm PEO, at both 4.5ºC and -19ºC, are consistent. They have low standard errors and there is not a significant difference between the tests on different dates as the experiment progressed. This shows that there is no significant degradation at either temperature for 500 ppm PEO, making it a good storage option for future use.

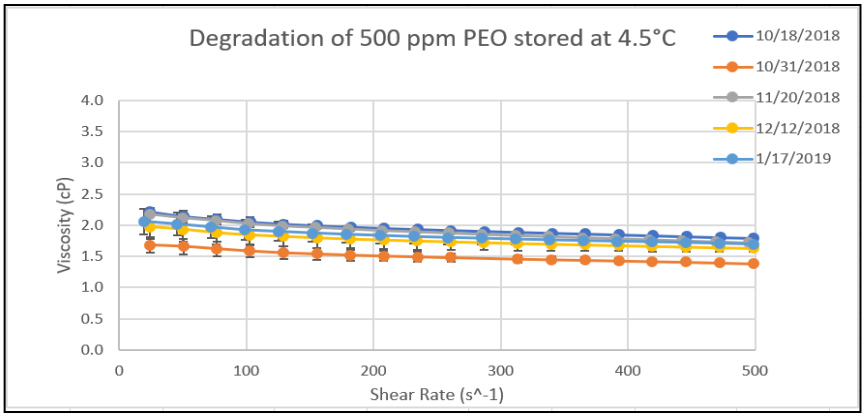


Figure 1. Effect of storage time on average viscosity (cP) of 500 ppm PEO stored at 4.5ºC.

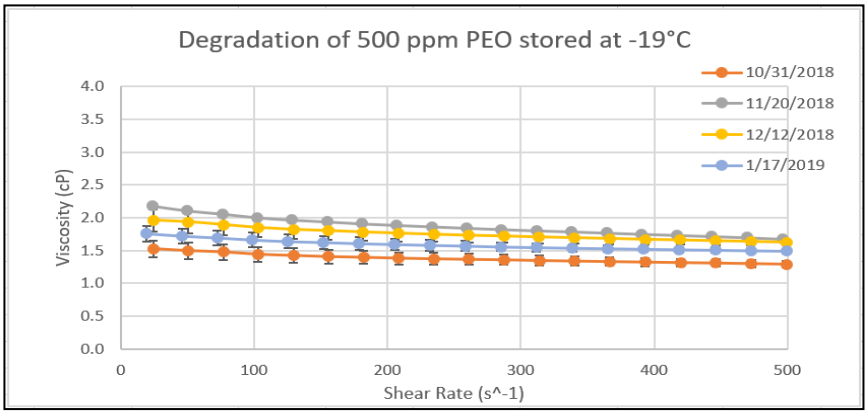


Figure 2. Effect of storage time on average viscosity (cP) of 500 ppm PEO stored at -19ºC.

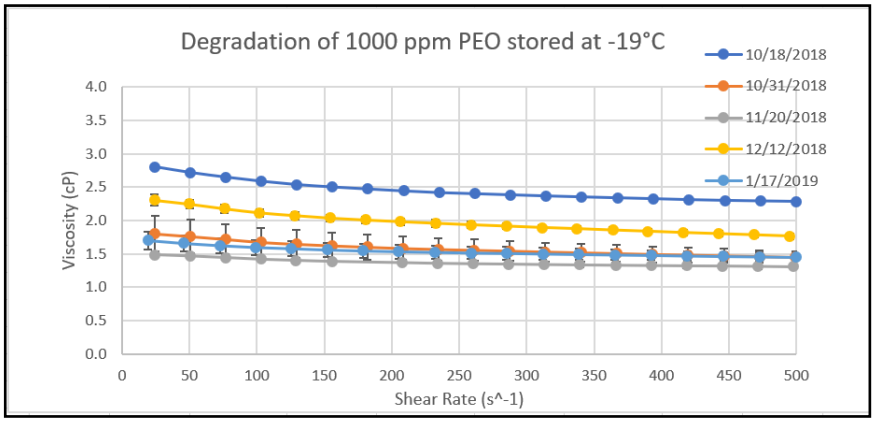


Figure 3. Effect of storage time on average viscosity (cP) of 1000 ppm PEO stored at -19ºC.

Very low concentrations of the high molecular weight (4000 – 4500 kDa) PEO of linear structure and water-soluble polymer is able to reduce the turbulent frictional drag of the water by as much as 80%. The flexibility of ether linkages combined with the extremely high molecular weight of water-soluble PEO produces solutions with elastic behavior. At high concentrations, with good lubricating, binding and film forming properties, PEO (POLYOX^{TM}) retards the release rate of drug/s and hence is widely used in pharmaceutical formulations like controlled release dosage forms, hot-melt technology and mucoadhesive dosage forms [; Ma L, Deng L, Chen J. Applications of poly(ethylene oxide) in controlled release tablet systems: a review. Drug Development and Industrial Pharmacy. 40(7):845-851; 2013]. PEO water-soluble molecules are nontoxic and have received FDA approvals for a number of food and drug applications. Aqueous solutions of PEO are environmentally degradable due to oxidation and aerobic biodegradation. Water-soluble PEO are nontoxic and have received FDA approvals for a number of applications [Shah AP and Bhandary SR. POLYOX (polyethylene oxide) - applications in pharma industry. Pharmaceutical Reviews 8(3) 2010].

The major problem of producing DRP solutions in advance is their mechanical stress- and storage time-related degradation, which currently makes it necessary to prepare the injectable solution from commercial DRP powder prior to each animal experiment. In this Sub-Aim we planned to develop and optimize a novel process of preparation, sterilization, and storage conditions for creation of the concentrated DRP-RF (PEO) solutions which would be used within a few minutes which will be needed for quick defrosting and dilution of the stored frozen concentrated solution when it is needed for treatment. The following parameters will be optimized to create DRP solutions which will have little or no degradation after dissolving, dialysis, sterilization, and frizzing storage: optimal DRP concentrations in solutions exposed to sterilization via filtration, variation of freezing temperatures and storage time, exposure to rapid freezing and thawing processes, and quick dilution to nanomolar concentrations for IV injections or as an additive to the resuscitation fluid.

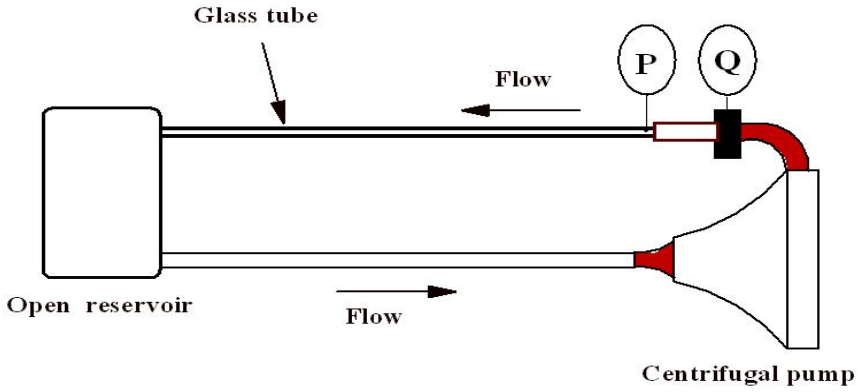
These solutions are tested in the turbulent flow system to confirm the polymer drag reducing properties (the most important physical properties of the DRP solutions), and in the measurement of polymer viscoelastic properties over a large range of shear rates relevant to those in vascular system.

We tested sterilization procedure on PEO to confirm the optimal concentration and filtration conditions to preserve drag reducing and viscoelastic properties of these polymers. Filtration was performed at -200 and -600 mmHg using the Millex Flip-Cup 0.22 um filters with a PEO concentration of 1500 ppm. Following the filtration experiments, we tested filtered solutions using a viscoelastometer (Vilastic) and compared results to the unfiltered polymer solutions. We found that filtration of PEO (linear molecular structure) solution had little or no effect on the drag-reducing ability and viscoelastic properties of the PEO after a short filtration process (about10 min)

Preparation of PEO solutions for optimizations and stability toward it storage: Since DRPs have a tendency to mechanical degradation over the time and due to exposure to high shear stress conditions, special care should be taken to prevent polymer degradation due to handling. The DRP-RF used in most of our in vivo and in vitro experiments, PEO with MW ~ 4000 kDa (Sigma-Aldrich, Saint Louis, MO). The powder of PEO was dissolved in sterile phosphate buffered saline (PBS) at concentrations of 4000 ppm and 1000 ppm. The solutions were tested in the turbulent flow system to verify drag-reducing efficiency.

Drag reduction test circuit: Mechanical degradation of drag reducing polymer molecules dissolved in fluid diminish their drag reducing ability. At the beginning experiments in this program, our lab has used pumps commonly used for clinical circulatory support (Centrifugal pump Medtronic BioMedicus) to induce shear mediated degradation of polymer solutions in a turbulent flow system. However, the shear profile within these pumps and the total amount of accumulated shear stress delivered to the polymer solution ultimately which leads to polymer degradation and reduced drag

reduction is not well characterized. Prior several month experiments comparing storage conditions at 4 ºC vs. -80 ºC and sample storage under Argon gas vs. room air yielded inconclusive results which we hypothesized to be due quick PEO degradation generated by the pump which prohibited recording accurate data collection on storage degradation.



New gravity driven turbulent flow system: This system is gravity-driven (ΔP =~200 mmHg) and consists of a tube with 0.25-inch inner diameter that produces turbulent flow with Reynolds numbers greater than 12,500 (development turbulence). Pressure and flow parameters are recorded for each run of the PEO solution, and drag reduction, as well as shear stress and shear rates, are calculated. The effect of the shear stress on the drag reducing ability is quantified and compared across solutions stored for different lengths of time. The reproducibility of this new method is also tested by comparing the effect the system has on PEO degradation each test day, keeping all other parameters equal.

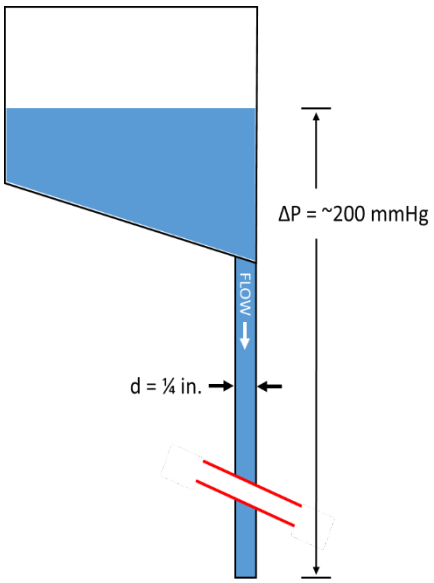


Figure 2

Each tested PEO solution was prepared by dilution of 4000 ppm and 1000 ppm to 10 ppm before it started to run through the turbulent system. Then, viscosity and elasticity of the original PEO solutions were measured using a Vilastic-3 viscoelasticity analyzer (Vilastic Inc, Austin, TX). Our current results indicate that there is no significant decrease of the PEO solution effectiveness as storage time increases. This indicates that refrigerating PEO solutions at either concentration may be viable long-term storage options. Each sample was run through the system 15 times and the time the

solution took to reach the beaker was measured. Drag reduction was calculated for each run using the following equation:

$$\boldsymbol{drag\ reduction\ (DR)\%} = \frac{\boldsymbol{time_{H2O} - time_{trial}}}{\boldsymbol{time_{H2O}}}$$

Degradation of PEO caused by turbulent flow is demonstrated in Tables 1 and 2 and in graphs presented the effect of number of runs on increase of flow time due to degradation of the PEO by exposure to turbulent flow.

Table 1: Drag reduction calculated for the first and last run of the 1000 ppm PEO

	Drag Reduction%				
Run #	Week 2	Week 3	Week 4	Week 5	Week 6
1	33.9	35.1	37.7	37.6	38.8
15	12.0	16.0	17.3	17.4	16.0

Table 2: Drag reduction calculated for the first and last run of the 4000 ppm PEO

	Drag Reduction%				
Run #	Week 2	Week 3	Week 4	Week 5	Week 6
1	34.9	32.0	37.6	37.0	38.1
15	12.8	16.0	14.8	16.4	19.3

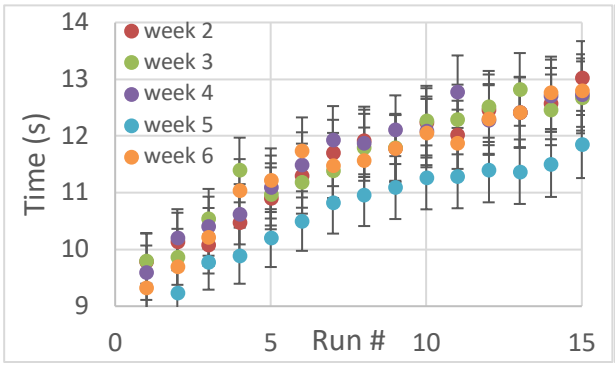


Figure 3: Effect of number of runs on time length of run the samples prepared from 4000 ppm PEO solution

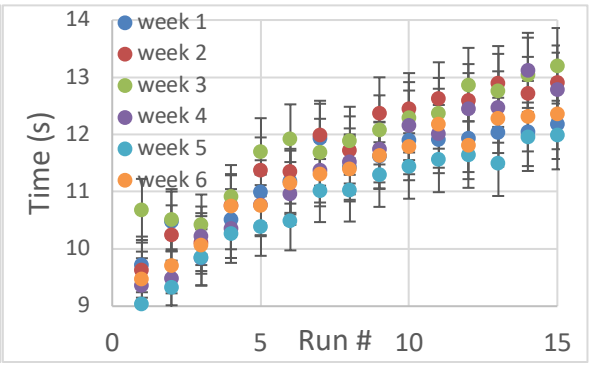


Figure 4: Effect of number of runs on time length of run the samples prepared from 1000 ppm PEO solution

PEO solutions characterization and storage: Polyethylene oxide solutions (4M MW, Sigma Aldrich, USA) at concentrations of 1000 ppm and 4000 ppm were prepared and their viscosity and elasticity were measured using the Vilastic-3 viscoelasticity analyzer (Vilastic Scientific, Inc., Austin, TX, USA) over shear rates range from 1 to 500 s^{-1} (presented in the graphs below). Higher repeatability using the Vilastic-3 instead of a Brookfield rheometer, with which prior storage tests were conducted will better depict changes in DRP due to storage degradation. Solutions of prepared mixtures were separated into aliquots and stored at 4ºC for preparation of our now ongoing storage degradation study using our new flow system. Samples will be measured weekly for DRP viscosity and elasticity as well as drag reducing ability (see below) to ensure sample viability.

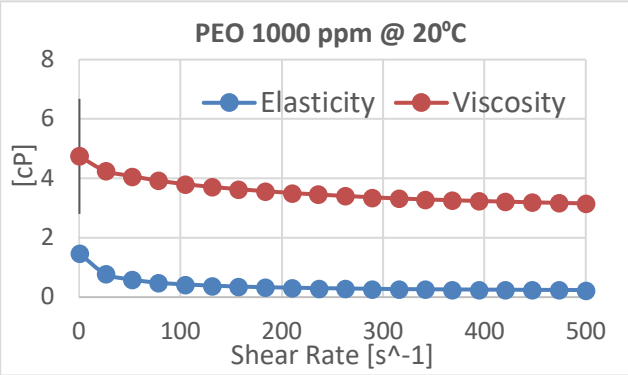


Figure 5: Viscosity and elasticity of the PEO-1000 solution recorded by Vilastic-3 viscoelasticity analyzer

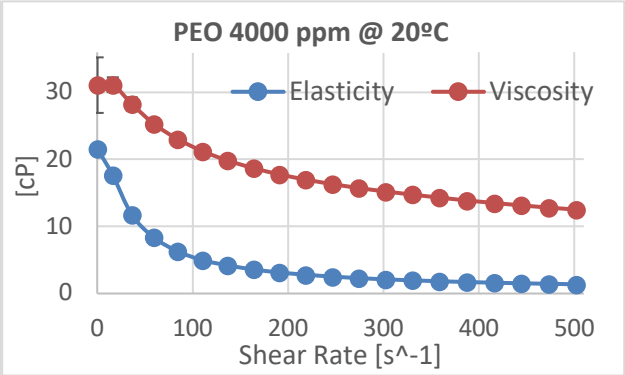
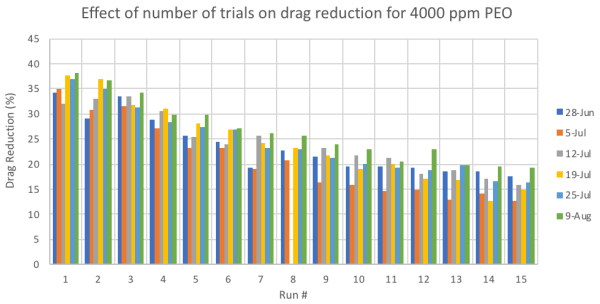
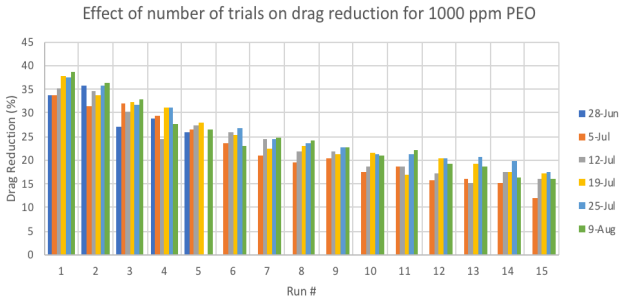


Figure 6: Viscosity and elasticity of the PEO-4000 solution recorded by Vilastic-3 viscoelasticity analyzer

The two pictures below demonstrate the diminished drag reduction during 15 runs of the samples obtained from PEO-4000 and PEO-1000 solutions which were stored over 5 weeks in refrigerator and were tested every week.





Conclusions: These experiments demonstrated that while PEO solutions of high concentration were not very sensitive to storage in refrigerator. The variation of a drag reduction at the first run is mostly related to the procedure steps from worming the sample to a room temperature and dilution for injection. To develop a strong protocol for producing the primary concentrated PEO solution and its storage, from which DRP-RF will be prepared and used without delay and with no degradation of the polymer molecules in solutions, we will continue to vary thickness/concentration of the original solutions to be frozen, and to optimize volume of the remedy samples.

What opportunities for training and professional development has the project provided?
If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

Nothing to Report

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

Local Public and Scientific Community:

1. Poster presentation at UNM HSC Neuroscience Day, Albuquerque, NM, 03/16/2018 – "Novel Advanced Resuscitation Fluid for TBI with HS"
2. Poster presentation at UNM HSC Neuroscience Day Albuquerque, NM, 03/17/2019 – "Neuroprotective Resuscitation Fluid for TBI with HS"

National and International Scientific Community:

1. Oral presentation at the Meeting of the International Society on Oxygen Transport to Tissue (ISOTT), Seoul, S. Korea, July 1-5, 2018 – "Resuscitation with drag reducing polymer after traumatic brain injury with hemorrhagic shock reduces microthrombosis and oxidative stress"
2. Poster presentation at the Joint Symposium of the International and National Neurotrauma Societies and AANS/CNS Section on Neurotrauma and Critical Care, Toronto, Canada, August 11-16, 2018: – "Resuscitation with drag reducing polymer reduces microthrombosis and oxidative stress after traumatic brain injury with hemorrhagic shock"
3. Oral Presentation at the Military Health System Research Symposium, Kissimmee, FL, August 20-23, 2018 – "A novel advanced resuscitation fluid with drag reducing polymer enhances cerebral microcirculation and tissue oxygenation after traumatic brain injury complicated by hemorrhagic shock"
4. Lead Poster Presentation at International Stroke Conference, Honolulu HI, 2019 – "Resuscitation Fluid With Drag Reducing Additive Reduces Microthrombosis and Oxidative Stress After Traumatic Brain Injury Complicated by Hemorrhagic Shock"

5. Lead Poster Presentation at National Neurotrauma Symposium, Pittsburgh, PA, 06.29-07.03, 2019 – "Neuroprotective role of drag reducing polymers additive to Hetastarch resuscitative fluid in rat model of TBI with hemorrhagic shock"
6. Oral Presentation and Poster at the 47th Annual Meeting of the International Society for Oxygen Transport to Tissue, July 27-31, 2019, Albuquerque, NM – "Drag reducing polymer addition to colloid resuscitation fluid enhances cerebral microcirculation and tissue oxygenation after traumatic brain injury complicated by hemorrhagic shock"
7. Poster Presentation at the 29th International Symposium on Cerebral Blood Flow, Metabolism and Function, July 4 – 7, 2019, Yokohama, Japan – "Microthrombosis and oxidative stress reduction by novel resuscitation fluid for TBI with HS"
8. Scientific Breakout Session Oral Presentation at the Military Health System Research Symposium, Kissimmee, FL, August 19-22, 2019 – "Drag Reducing Polymer Hetastarch Resuscitation Fluid Improves Cerebral Microcirculation, Tissue Oxygenation, And Neuronal Survival By Reducing Microthrombosis And Oxidative Stress After Traumatic Brain Injury Complicated By Hemorrhagic Shock"
9. Poster Presentation at the International Symposium on Intracranial Pressure and Neuromonitoring, September.08-11, 2019-ICP, Lewen, Belgium – "Improved Cerebral Perfusion Pressure and Microcirculation by Drag Reducing Polymer-Enforced Resuscitation Fluid after TBI and Hemorrhagic Shock"

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "Nothing to Report."
Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

To obtain all approvals to continue the work and to complete the project by 09/14/2021.

4. IMPACT: Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

During this year we obtained and expanded principal evidences of neuroprotective efficiency of our DRP-enhanced resuscitation fluid and compared colloid, crystalloid and hypertonic fluids.

We have also developed, for the first time, in-vivo two-photon imaging of superoxide (hydroethidine) in a rat cortex. Previous studies we performed on post-mortem sections.

Further study will lead to the pre-clinical studies on swine and to the development to the new, neuroprotective strategy of field resuscitation in patients with TBI and HS due to polytrauma.

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

Nothing to Report

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to Report

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to Report

5. **CHANGES/PROBLEMS:** The Project Director/Principal Investigator (PD/PI) is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:

Changes in approach and reasons for change
Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

No significant changes anticipated

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

No problems encountered

Changes that had a significant impact on expenditures
Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

We had a several changes during reporting period that affected expenditures resulting in less cost than anticipated. Among them is a 3 months outage of the MRI.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

N/A

Significant changes in use or care of vertebrate animals.

No changes

Significant changes in use of biohazards and/or select agents

No changes

6. **PRODUCTS:** List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."

- **Publications, conference papers, and presentations**

Report only the major publication(s) resulting from the work under this award.

Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Bragin DE, Bragina OA, Kameneva MV, Nemoto EM. Resuscitation with Drag Reducing Polymers after Traumatic Brain Injury with Hemorrhagic Shock Reduces Microthrombosis and Oxidative Stress. Adv Exp Med Biol. 2020; 1232:39-45. doi: 10.1007/978-3-030-34461-0_6. PubMed PMID: 31893392.

Bragin DE, Lara DA, Bragina OA, Kameneva MV, Nemoto EM. Resuscitation Fluid with Drag Reducing Polymer Enhances Cerebral Microcirculation and Tissue Oxygenation After Traumatic Brain Injury Complicated by Hemorrhagic Shock. Adv Exp Med Biol. 2018; 1072:39-43. doi: 10.1007/978-3-319-91287-5_7. PubMed PMID: 30178321.

D.E. Bragin, O.A. Bragina, L. Berliba, M.V. Kameneva and E.M. Nemoto. Drag reducing polymer addition to colloid resuscitation fluid enhances cerebral microcirculation and tissue oxygenation after traumatic brain injury complicated by hemorrhagic shock, *In Print*.

Denis E Bragin, Lucy Berliba, Olga A Bragina, Marina Kameneva, Edwin M Nemoto. Improved Cerebral Perfusion Pressure and Microcirculation with Drag Reducing Polymer-Enforced Resuscitation Fluid after TBI and Hemorrhagic Shock, *Acta Neurochirurgica*, *In Print*.

The manuscript comparing colloid based vs. crystalloid DRP-added resuscitation fluid is under revision.

The manuscript, describing the beneficial effects of advanced resuscitation fluid on cerebral microcirculation and metabolism in TBI with HS is under revision.

Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: Author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to Report

Other publications, conference papers, and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

Abstracts

1. D. Bragin, D, Lara, O. Bragina, M. Kameneva, E. Nemoto. Novel Advanced Resuscitation Fluid for TBI with HS, Abstract Book for UNM Neuroscience Day, Albuquerque, NM, 03/16/2018, P. 31.

2. D. Bragin, O. Bragina, L. Berliba, M. Kameneva, E. Nemoto. Resuscitation with drag reducing polymer after traumatic brain injury with hemorrhagic shock reduces microthrombosis and oxidative stress, Abstract Book for the International Society on Oxygen Transport to Tissue, 2018, P. 49.
3. D. Bragin, O, Bragina, M. Kameneva, E. Nemoto. Resuscitation with drag reducing polymer reduces microthrombosis and oxidative stress after traumatic brain injury with hemorrhagic shock, J. Neurotrauma, 35, A-136. (Abstracts for National Neurotrauma Symposium 2018).
4. D.E. Bragin, O.A. Bragina, L. Berliba, M.V. Kameneva, E.M. Nemoto. A novel neuroprotective resuscitation fluid for traumatic brain injury with hemorrhagic shock, Neurocritical Care, Abstracts for Neurocritical Care Society 16th Annual Meeting 2018, 29: S7.
5. D.E. Bragin, O.A. Bragina, L. Berliba, M.V. Kameneva and E.M. Nemoto. A novel advanced resuscitation fluid with drag reducing polymer enhances cerebral microcirculation and tissue oxygenation after traumatic brain injury complicated by hemorrhagic shock. Abstract fo the Military Health System Research Symposium 2018.
6. Denis E Bragin, Olga A Bragina, Lusy Berliba, Edwin M Nemoto. Resuscitation Fluid With Drag Reducing Additive Reduces Microthrombosis and Oxidative Stress After Traumatic Brain Injury Complicated by Hemorrhagic Shock, Stroke, Abstract for the International Stroke Conference, 2019, Volume 50, Issue Supp-1, WMP74:
7. D. Bragin, D, Lara, O. Bragina, M. Kameneva, E. Nemoto. Neuroprotective Resuscitation Fluid for TBI with HS, Abstract Book for UNM Neuroscience Day, Albuquerque, NM, 03/17/2019, P. 40.
8. Denis Bragin, Olga Bragina, Lucy Berliba, Marina Kameneva, Edwin Nemoto. Neuroprotective role of drag reducing polymers additive to Hetastarch resuscitation fluid for TBI with hemorrhagic shock, J. Neurotrauma, 2019, 36, A67-68.
9. D. Bragin, O. Bragina, L. Berliba, M. Kameneva and E. Nemoto. Microthrombosis and oxidative stress reduction by novel resuscitation fluid for traumatic brain injury with hemorrhagic shock, JCBFM, 2019, Vol. 39(1S), P. 309.
10. D.E. Bragin, O.A. Bragina, L. Berliba, M.V. Kameneva and E.M. Nemoto. Drag reducing polymer addition to colloid resuscitation fluid enhances cerebral microcirculation and tissue oxygenation after traumatic brain injury complicated by hemorrhagic shock, Abstract book for the 47th Annual Meeting of the International Society on Oxygen Transport to Tissue, 2019, P. 93.

11. D.E. Bragin, O.A. Bragina, L. Berliba, M.V. Kameneva and E.M. Nemoto. Drag Reducing Polymer Hetastarch Resuscitation Fluid Improves Cerebral Microcirculation, Tissue Oxygenation, And Neuronal Survival By Reducing Microthrombosis And Oxidative Stress After Traumatic Brain Injury Complicated By Hemorrhagic Shock. Abstracts for the Military Health System Research Symposium, Kissimmee, FL, August 19-22, 2019.
12. D.E. Bragin, O.A. Bragina, L. Berliba, M.V. Kameneva and E.M. Nemoto. Improved Cerebral Perfusion Pressure and Microcirculation by Drag Reducing Polymer-Enforced Resuscitation Fluid after TBI and Hemorrhagic Shock, Electronic Abstracts for the International Symposium on Intracranial Pressure and Neuromonitoring, September.08-11, 2019-ICP, Lewen, Belgium.

Scientific Conferences Presentations:

1. Poster presentation at UNM HSC Neuroscience Day, Albuquerque, NM, 03/16/2018 – "Novel Advanced Resuscitation Fluid for TBI with HS"
2. Poster presentation at UNM HSC Neuroscience Day Albuquerque, NM, 03/17/2019 – "Neuroprotective Resuscitation Fluid for TBI with HS"
3. Oral presentation at the Meeting of the International Society on Oxygen Transport to Tissue (ISOTT), Seoul, S. Korea, July 1-5, 2018 – "Resuscitation with drag reducing polymer after traumatic brain injury with hemorrhagic shock reduces microthrombosis and oxidative stress"
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- **Website(s) or other Internet site(s)**
  List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

  Nothing to Report

- **Technologies or techniques**
  Identify technologies or techniques that resulted from the research activities. In addition to a description of the technologies or techniques, describe how they will be shared.

  Nothing to Report

- **Inventions, patent applications, and/or licenses**
  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. State whether an application is provisional or non-provisional and indicate the application number. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

  Nothing to Report

- **Other Products**
  Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment, and/or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:
  - *data or databases;*
  - *biospecimen collections;*
  - *audio or video products;*
  - *software;*
  - *models;*
  - *educational aids or curricula;*
  - *instruments or equipment;*
  - *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
  - *clinical interventions;*
  - *new business creation; and*
  - *other.*

  Nothing to Report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change."

Name:	*Denis Bragin*
Project Role:	*PI*
Nearest person month worked:	*5 (0 due to fund freeze)*
Contribution to Project:	*Dr. Bragin performed optical imaging, data interpretation and manuscripts preparation.*
Funding Support:	*NIH-NINDS 1 R01 NS112808*
Name:	*Afshin Divani –*
Project Role:	*PROPOSED NEW SITE PI*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*1 (0 funded)*
Contribution to Project:	*Overseen the project.*
Name:	*Edwin Nemoto*
Project Role:	*Co-investigator*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*0.0 (Retired Professor)*
Contribution to Project:	*Dr. Nemoto has performed data analysis and interpretation.*
Funding Support:	
Name:	*Tongsheng Zhang*
Project Role:	*Investigator*
Nearest person month worked:	*1.2 (0 due to fund freeze)*
Contribution to Project:	*Dr. Zhang has performed data analysis.*
Funding Support:	*Albuquerque Magnetic Resonance, Inc*
Name:	*Lucy Berliba*
Project Role:	*Research Specialist*
Nearest person month worked:	*7.2 (0 due to fund freeze)*
Contribution to Project:	*Mrs. Berliba has performed rat surgeries and physiological monitoring.*
Name:	*Olga Bragina*
Project Role:	*Sr. Research Specialist*
Nearest person month worked:	*0.0*
Contribution to Project:	*She has performed histochemistry.*

Funding Support:

Sub-award

Name:	*Marina Kameneva*
Project Role:	*Sub-award PI*
Nearest person month worked:	*1.2*
Contribution to Project:	*Involved in development and testing of a novel process of preparation and storage conditions for DRP solutions.*
Funding Support:	*NIH-NHLBI R01 HL089456* *Commonwealth of PA.*
Name:	*Sarah Tolaymat*
Project Role:	*Undergraduate Student Researcher*
Nearest person month worked:	*6*
Contribution to Project:	*Involved in development and testing of a novel process of preparation and storage conditions for DRP solutions.*

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

Dr. Zhang has retired.

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.
Provide the following information for each partnership:
Organization Name:
Location of Organization: (if foreign location list country)
Partner's contribution to the project (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

Nothing to Report

8. SPECIAL REPORTING REQUIREMENTS

QUAD CHARTS: If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.

A Novel Advanced Resuscitation Fluid for Traumatic Brain Injury with Hemorrhagic Shock

Log Number: DM160142

Award Number: W81XWH-17-2-0053

PI: Bragin, D.E. **Org:** Dept. of Neurosurgery, University of New Mexico School of Medicine/ McGowan Inst. for Regenerative Medicine, University of Pittsburgh (Sub-contractor)

Award Amount: $1,416,397.00

Study/Product Aim(s)

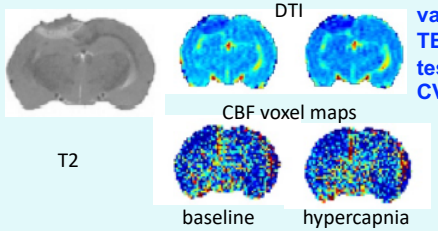
Current resuscitation fluids for traumatic brain injury with hemorrhagic shock (TBI/HS) do not ameliorate impaired cerebral microvascular flow leading to hypoxia, neuronal death, increased mortality and poor neurological outcome. Nanomolar concentrations of blood soluble intravenous drag reducing polymers (DRP) improve cerebral microcirculation and tissue oxygenation. The proposed research aims to ameliorate impaired cerebral and systemic microcirculation by restoring capillary perfusion after TBI/HS using novel, advanced by DRP addition, resuscitation fluid.

Hypothesis: Addition of DRP to resuscitation fluid (DRP-RF) for TBI/HS will attenuate the severity of injury, increase survival, improve neurologic recovery and reduce the volume of fluid required to prevent the transition of HS to the irreversible stage or functional impairment of the brain.

- Specific Aim 1: Demonstrate the major mechanisms and the acute, beneficial effects of DRP-RF for up to 8 hours after TBI/HS compared to crystalloid or colloid fluids on both the brain and systemic microcirculation, metabolism and pathology.
- Specific Aim 2: Prove the beneficial effects of DRP-RF on long-term recovery and neurologic outcome comparing with crystalloid and colloid fluids for up to 4 weeks after TBI/HS.

Approach

- Using rat fluid percussion injury for TBI in rats, we will evaluate the beneficial effects of DRP-RF brain circulation, metabolism and neuronal survival in acute phase of TBI/HS (up to 8 hrs.) by in-vivo Laser Speckle Contrast Imaging and Two-photon Laser Scanning Microscopy.
- Long term anatomical and neurological outcome will be evaluated for up to 4 wks. after TBI/HS by magnetic resonance imaging, behavioral tests and histochemistry.



Representative MRI images showing cerebral vascular reactivity (CVRx) changes after TBI/HS measured by MRI and hypercapnia test. Resuscitation with DRP better preserved CVRx.

For the reported period, we have completed Specific Aim 1 – Elucidation of the major mechanisms and beneficial effects of DRP resuscitation fluid (DRP-RF) infused in animals at the "pre-hospital" acute phase up to 6 hours after traumatic brain injury with hemorrhagic shock (TBI/HS) compared to crystalloid, colloid or hypertonic fluids in controls and contrasted to HS or TBI only using sham as a control. Currently we are working on the Specific Aim 2: (Compare the beneficial effects of DRP-RF on long-term recovery and neurologic outcomes compared to crystalloid, colloid and hypertonic fluid treatments for up to 4 weeks after TBI/HS. Sub-Contractor performed experiments on DRP characterization and storage and drag reduction test circuit development

Timeline and Cost

Activities / CY	17	18	19	20
Specific Aim 1: Demonstrate the major mechanisms and the acute, beneficial effects of DRP-RF for up to 8 hours after TBI/HS compared to crystalloid or colloid fluids on both the brain, systemic microcirculation, metabolism and pathology.				
Specific Aim 2: Prove the beneficial effects of DRP-RF on long-term recovery and neurologic outcome comparing with crystalloid and colloid fluids for up to 4 weeks after TBI/HS.				
Estimated Budget ($1,416K)	**$195K**	**$455K**	**$457K**	**$309K**

Updated: 10/15/2020

Goals/Milestones

CY17 Goal– Specific Aim 1: ☑ Acute effects of DRP-RF (up to 8 hrs.). ☑ Brain microvascular circulation and tissue oxygenation (DRP vs. crystalloids

CY18 Goals – Specific Aim 2: ☑ Long term neurological and anatomical recovery (up to 4 weeks). ☑ Continuation of Specific Aim 1.

CY19 Goal – ☑ Completion of Specific Aim 1, ☑ Continuation of Specific Aim 2, data interpretation and transition to translational phase of research (pre-clinical and clinical studies)

CY20 Goal – Completion of Specific Aim 2, data interpretation and transition to translational phase of research (pre-clinical and clinical studies)

Comments/Challenges/Issues/Concerns

- No changes/No concerns

Budget Expenditure to Date

Projected Expenditure: $1,416K

Actual Expenditure: $1,100K