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AWARD NUMBER: W81XWH-17-2-0053

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
Albuquerque, NM, 87131

REPORT DATE: October 2019

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
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REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

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1. REPORT DATE October 2019		2. REPORT TYPE Annual		3. DATES COVERED 09/15/2018-09/14/2019	
4. TITLE AND SUBTITLE A Novel Advanced Resuscitation Fluid for Traumatic Brain Injury with Hemorrhagic Shock				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER W81XWH-17-2-0053	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Denis E Bragin E-Mail: dbragin@salud.unm.edu				5d. PROJECT NUMBE	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) University of New Mexico Health Sciences Center 1 University of New Mexico, MSC 10 6515, Neurosurgery, Albuquerque, NM, 87131, USA				8. PERFORMING ORGANIZATION REPORT NUMBER	
McGowan Institute for Regenerative Medicine, University of Pittsburgh 450 Technology Drive, Suite 300 Pittsburgh, Pennsylvania 15219, USA					
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited					
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.					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	34	19b. TELEPHONE NUMBER (include area code)

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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 – 95%

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 – 90%

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 – 85%.

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 – 70%

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 – 20%

Subtask 2. Cognitive deficits (Passive avoidance - learning, Y- maze working and Novel Object - recognition memory) – 20-32 months – 25%.

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 – 20%

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

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

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 – 15%
Subtask 2. Capillary density evaluation by alkaline phosphatase staining – 20-32 months – 20%.
Subtask 3. Inflammation evaluations (microglia and astrocytes activation) – 20-32 months – 20%

Major Tasks 4: Data analysis and interpretation for Specific Aim 2 – 24-36 months – 20%

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 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 $38 \pm 0.5^\circ\text{C}$. 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.

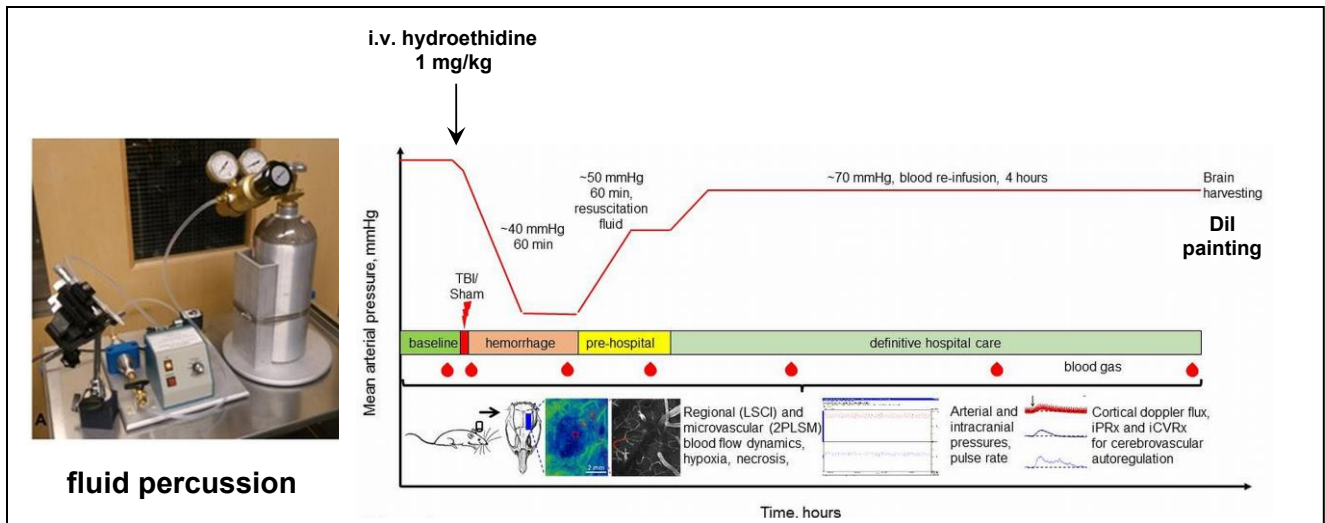


Fig. 1 Experimental Protocol

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.

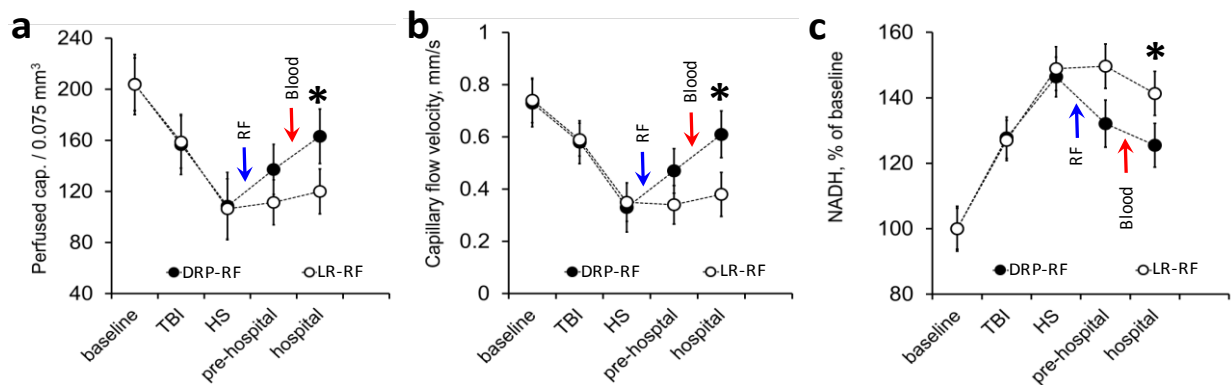


Fig. 2 Resuscitation with crystalloid based DRP-RF improves cerebral microvascular perfusion and tissue oxygenation impaired by TBI/HS, as shown by a) increased number of perfused capillaries; b) increased capillary flow velocity; c) increased tissue oxygenation (NADH decrease). Mean \pm SEM, N=10 rats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

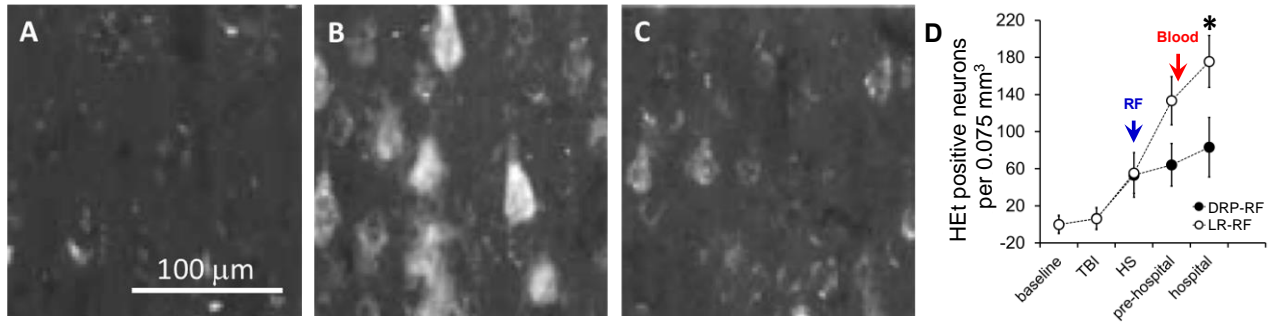


Fig. 3 Resuscitation with DRP-RF reduces superoxide production in cortical neurons after TBI with HS: a) Representative image of a rat cortex at baseline without ET positive neurons; b) Neurons with diffuse cytosolic ET fluorescence in a rat cortex from LR-RF group by the end of experiment; c) and from DRP-RF group; The dynamics of the increase in ET positive cortical neurons. Mean \pm 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.

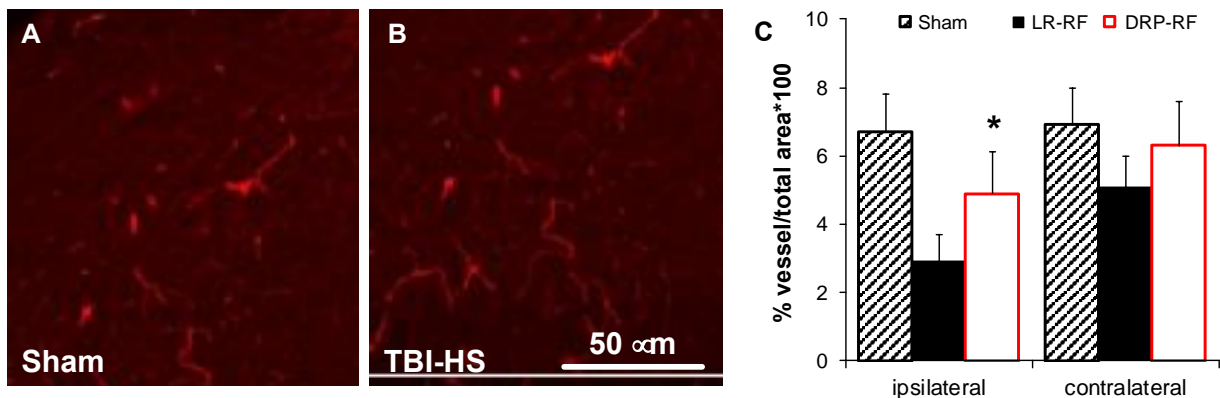


Fig. 4 Resuscitation with DRP-RF reduces microthrombosis in both hemispheres after TBI with HS as shown by post-mortem DiI vascular painting. a) Cortical microvascular network in Sham mouse brain; and b) after TBI with HS; c) Graph showing reduced cortical microvasculature in LR-RF group and better-preserved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

To evaluate *neuronal survival*, 200 μ L 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.

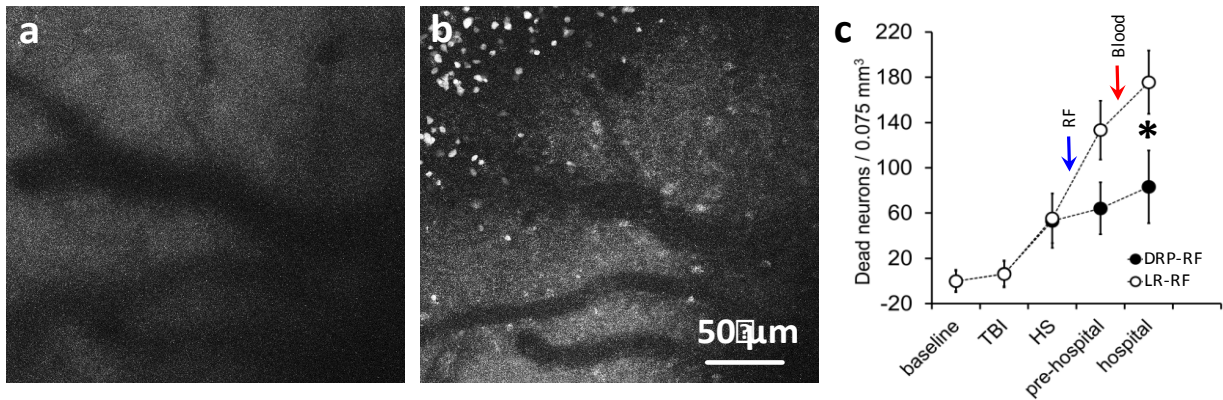


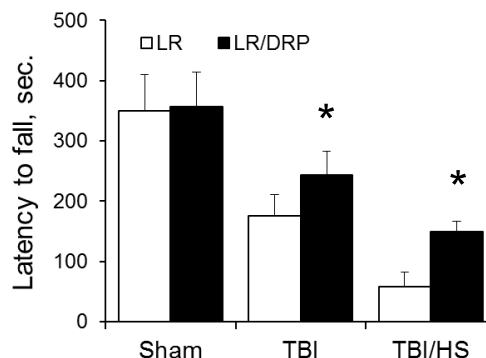
Fig. 5 Resuscitation with DRP-RF is neuroprotective: a) 2PLSM image of a rat cortex at baseline without dead neurons; b) Propidium Iodide stains neurons with damaged membranes reflecting necrosis of neurons after TBI/HS; c) DRP-RF protects neurons from necrosis (*= $P < 0.05$). Mean \pm SEM, N=10 rats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

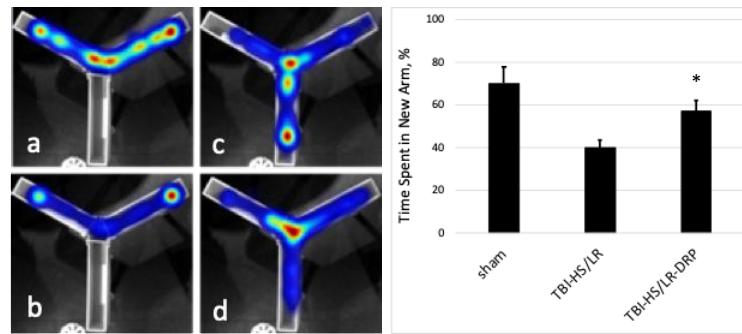
We have been working on the Specific Aim 2: (Compare the beneficial effects of DRP-RF on long-term recovery and neurologic outcomes compared to crystalloid, colloid and hypertonic fluid treatments for up to 4 weeks after TBI/HS).

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).

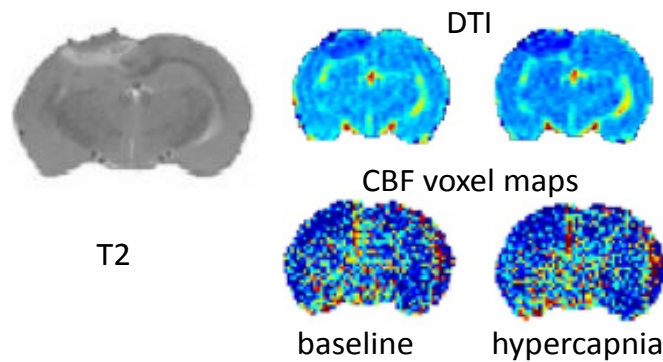
In particular we have shown the dynamic of CBF during 4 weeks after TBI/HS and the effect of DRP enforcement of resuscitation fluids using MRI. Behavioral testing revealed improvement in neurologic outcome. Some of the results are presented below:



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.



DRP 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.



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 lab is in the process of moving to the Lovelace Biomedical Research (Albuquerque, NM) to perform preliminary studies on Yucatan swine in collaboration with Raymond G. Murphy VA Medical Center (Albuquerque, NM) for the preparation of Investigational New Drug Application for submission to FDA.

The sub-awardee continued working on 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 (ongoing and future):

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.

Current 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 -20°C, 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.

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 (20°C), 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/cm²) 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 μm sterile filters. DRP solutions at 2000 ppm were driven at a rate of 2 mL/min through either polyvinylidene 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.

Future work: Light degradation, mechanical degradation, and sterilization testing of various concentrations of DRP are all underway. Additional testing using known chemical agents to degrade and reduce the viscosity of PEO is also being explored; it is well known that oxidizing agents such as hydrogen peroxide cause chain scission on DRP molecules.



Figure 1. Vilastic Viscoelasticity Analyzer

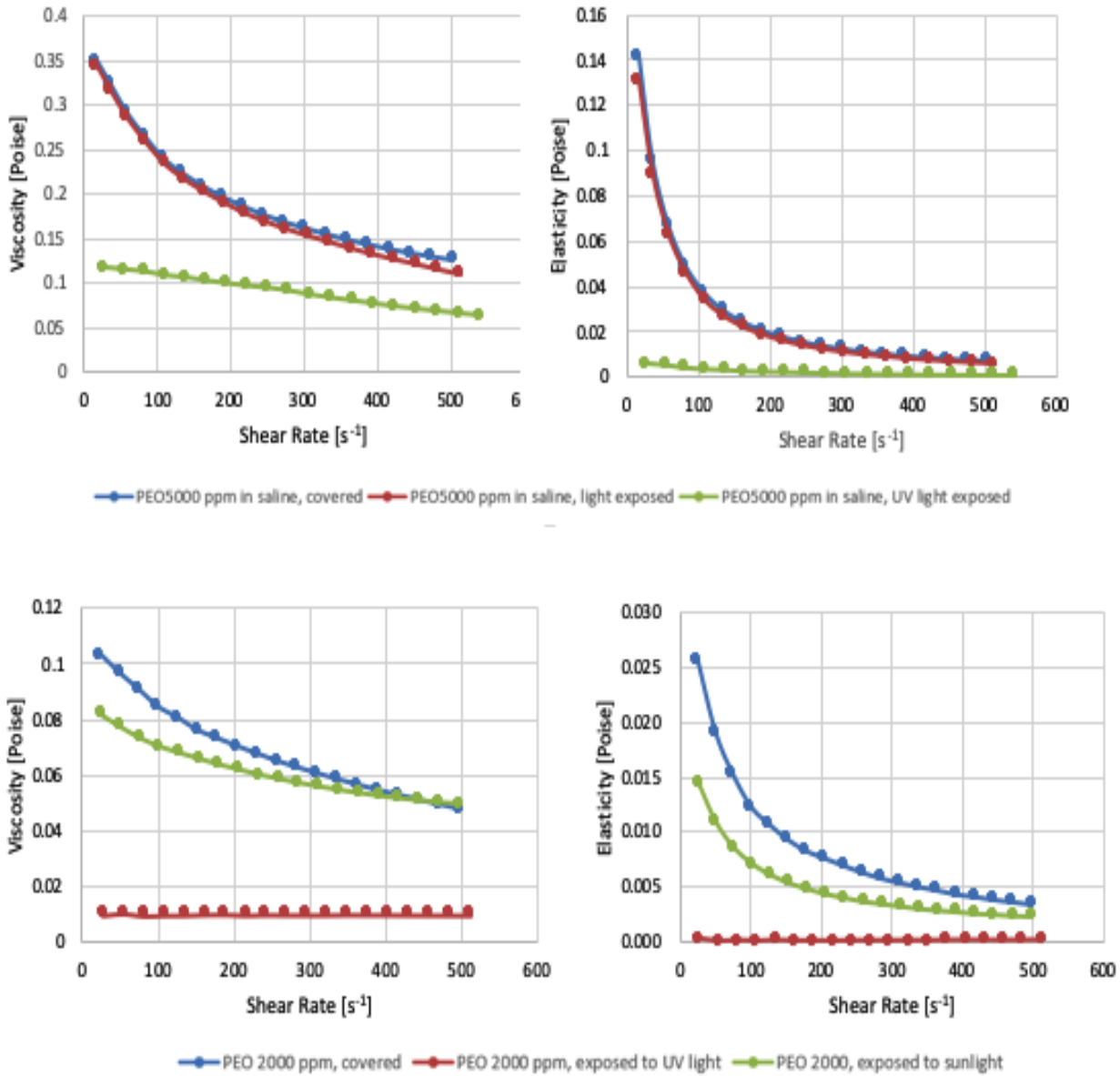


Figure 2. Viscosity and elasticity of PEO 5000 ppm solutions after exposure to no light (blue), grow light (red), and UV light (green) (TOP). Viscosity and elasticity of PEO 2000 ppm solutions after exposure to no light (blue), sunlight (green), and UV light (red) (BOTTOM).

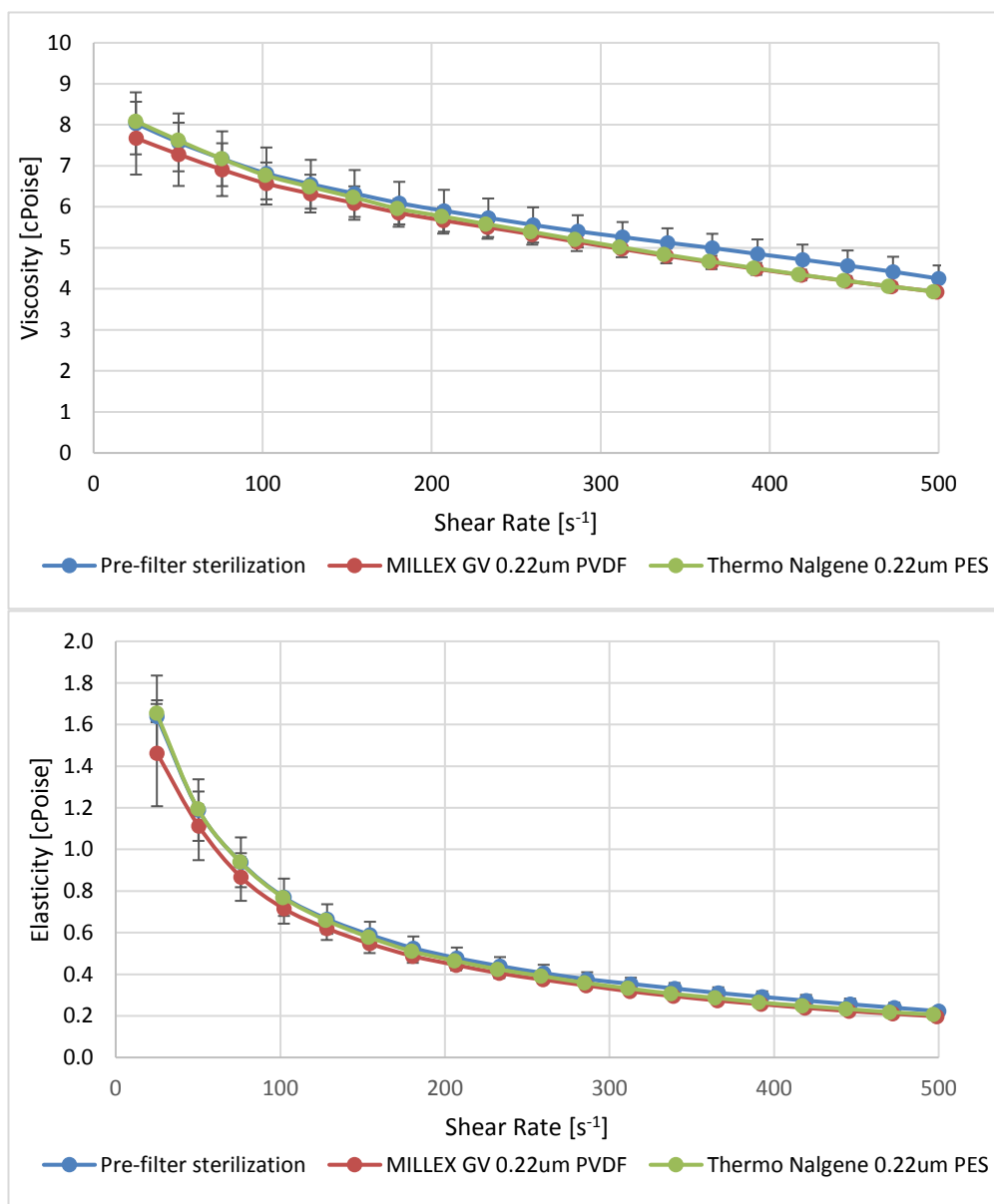


Figure 3. Viscosity (top) and elasticity (bottom) of 2000 ppm PEO solutions following sterilization of 0.22 filter membranes.

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:

Poster presentation at UNM HSC Neuroscience Day, Albuquerque, NM, 03/17/2019 – “Neuroprotective Resuscitation Fluid for TBI with HS”

National and International Scientific Community:

Oral presentation: “D.E. Bragin, O.A. Bragina, L. Berliba, M.V. Kameneva and E.M. Nemoto. A novel neuroprotective resuscitation fluid for traumatic brain injury with hemorrhagic shock.” was presented on the Neurocritical Care Society’s Annual Meeting 2018, September 25-28, Boca Raton, FL.

Poster “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” was presented at International Stroke Conference, Honolulu, HI, 02.06-02.08, 2019.

Lead poster presentation “Denis E Bragin, Olga A Bragina, Lusy Berliba, Edwin M Nemoto. Neuroprotective role of drag reducing polymers additive to Hetastarch resuscitative fluid in rat model of TBI with hemorrhagic shock” was presented at National Neurotrauma Symposium, Pittsburgh, PA, 06.29-07.03, 2019.

Poster “Denis E Bragin, Olga A Bragina, Lusy Berliba, M.V. Kameneva, Edwin M Nemoto. Microthrombosis and oxidative stress reduction by novel resuscitation fluid for TBI with HS” was presented at the 29th International Symposium on Cerebral Blood Flow, Metabolism and Function, July 4 – 7, 2019, Yokohama, Japan.

Poster “Denis E Bragin, Olga A Bragina, Lusy Berliba, M.V. Kameneva, Edwin M Nemoto, Drag reducing polymer addition to colloid resuscitation fluid enhances cerebral microcirculation and tissue oxygenation after traumatic brain injury complicated by hemorrhagic shock.” Was presented at the 47th Annual Meeting of the International Society for Oxygen Transport to Tissue, July 27-31, 2019, Albuquerque, NM, USA

Oral presentation “Denis E Bragin, Olga A Bragina, Lusy Berliba, M.V. Kameneva, Edwin M Nemoto, Enhanced By 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” was presented at the scientific breakout session Military Health System Research Symposium, August 19-22, Kissimmee, FL

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

To continue working on specified tasks according to SOW. I plan to complete all the tasks by the end of the next period.

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 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.*

By presenting our results on local, national and international conferences we improved public knowledge about novel resuscitation strategy we are developing in in lay and scientific, as well as civilian and military communities

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, 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, D.A. Lara, O.A. Bragina, M.V. Kameneva and E.M. Nemoto. Resuscitation with Drag Reducing Polymer after Traumatic Brain Injury with Hemorrhagic Shock Reduces Microthrombosis and Oxidative Stress, *Adv Exp Med Biol.* In Print, 2019

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, *Adv Exp Med Biol.* Accepted, August 2019.

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*, Submitted, September 2019.

The manuscript comparing colloid based vs. crystalloid DRP-added resuscitation fluid is in preparation.

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

- 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.
- 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:
- 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.
- 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, 36, A67-68.
- 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.
- 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, P. 93.

Conference Presentations:

- Oral presentation: “D.E. Bragin, O.A. Bragina, L. Berliba, M.V. Kameneva and E.M. Nemoto. A novel neuroprotective resuscitation fluid for traumatic brain injury with hemorrhagic shock.” was presented on the Neurocritical Care Society’s Annual Meeting 2018, September 25-28, Boca Raton, FL.
- Poster “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” was presented at International Stroke Conference, Honolulu, HI, 02.06-02.08, 2019.
- Lead poster presentation “Denis E Bragin, Olga A Bragina, Lusy Berliba, Edwin M Nemoto. Neuroprotective role of drag reducing polymers additive to Hetastarch resuscitative fluid in rat model of TBI with hemorrhagic shock” was presented at National Neurotrauma Symposium, Pittsburgh, PA, 06.29-07.03, 2019.

- Poster “Denis E Bragin, Olga A Bragina, Lusy Berliba, M.V. Kameneva, Edwin M Nemoto. Microthrombosis and oxidative stress reduction by novel resuscitation fluid for TBI with HS” was presented at the 29th International Symposium on Cerebral Blood Flow, Metabolism and Function, July 4 – 7, 2019, Yokohama, Japan.
- Poster “Denis E Bragin, Olga A Bragina, Lusy Berliba, M.V. Kameneva, Edwin M Nemoto, Drag reducing polymer addition to colloid resuscitation fluid enhances cerebral microcirculation and tissue oxygenation after traumatic brain injury complicated by hemorrhagic shock.” Was presented at the 47th Annual Meeting of the International Society for Oxygen Transport to Tissue, July 27-31, 2019, Albuquerque, NM, USA
- Oral presentation “Denis E Bragin, Olga A Bragina, Lusy Berliba, M.V. Kameneva, Edwin M Nemoto, Enhanced By 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” was presented at the scientific breakout session Military Health System Research Symposium, August 19-22, Kissimmee, FL

- **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.”

<i>Name:</i>	<i>Denis Bragin</i>
<i>Project Role:</i>	<i>PI</i>
<i>Nearest person month worked:</i>	<i>5</i>
<i>Contribution to Project:</i>	<i>Dr. Bragin performed optical imaging, data interpretation and manuscripts preparation.</i>
<i>Funding Support:</i>	<i>NIH-NIGMS P20 GM109089 NIH-NINDS 1 R01 NS112808</i>

<i>Name:</i>	<i>Edwin Nemoto</i>
<i>Project Role:</i>	<i>Co-investigator</i>
<i>Researcher Identifier (e.g. ORCID ID):</i>	
<i>Nearest person month worked:</i>	<i>0.6</i>
<i>Contribution to Project:</i>	<i>Dr. Nemoto has performed data analysis and interpretation.</i>
<i>Funding Support:</i>	<i>NIH-NIGMS P20 GM109089 NIH-NINDS 1 R01 NS112808 Neurosurgery Department Fund</i>

<i>Name:</i>	<i>Tongsheng Zhang</i>
<i>Project Role:</i>	<i>Investigator</i>
<i>Nearest person month worked:</i>	<i>1.2</i>
<i>Contribution to Project:</i>	<i>Dr. Zhang has performed data analysis.</i>
<i>Funding Support:</i>	<i>Albuquerque Magnetic Resonance, Inc</i>

Name: Lucy Berliba
Project Role: Research Specialist
Nearest person month worked: 7.2
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: NIH-NIGMS P20 GM109089
Neurosurgery Department Fund

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. Bragin currently has only DOD grant where he has 5-person months effort. It would be helpful to increase his effort on this grant for boosting the study.

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)

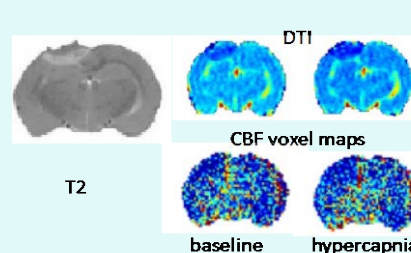
Current resuscitation fluids for traumatic brain injury with hemorrhagic shock (TBI/HS) do not ameliorate impaired cerebral microvascular flow leading to hypoxia, neuronal death, increased mortality and poor neurological outcome. Nanomolar concentrations of blood soluble intravenous drug reducing polymers (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 drug 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.		[Gantt bar spanning CY 17, 18, and 19]			
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.			[Gantt bar spanning CY 18 and 19]		[Gantt bar in CY 20]
Estimated Budget (\$1,416K)		\$195K	\$455K	\$457K	\$309K

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: \$750K

Updated: 03/30/2018

9. APPENDICES:

