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PRINCIPAL INVESTIGATOR: Afshin Divani

RECIPIENT: University of New Mexico Health Sciences Center
1 University of New Mexico,
MSC 08 4720, Neurology,
Albuquerque, NM, 87131-0001, USA

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Afshin Divani

E-Mail: ADivani@salud.unm.edu

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University of New Mexico Health Sciences Center
1 University of New Mexico, MSC 08 4720, Neurology, Albuquerque, NM, 87131-0001, USA

Lovelace Biomedical Research Institute
2425 Ridgecrest Dr. SE, Albuquerque, NM 87108-5127, USA

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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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TABLE OF CONTENTS

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%

Subtask 3. H&E, Fluoro Jade C and Cresyl Violet to identify dead vs. alive neurons - 12-30 months 20-32 – 100% (dead vs. alive neurons were evaluated by in-vivo propidium iodide, staining's will be completed with Specific Aim 2, Major task 3 for better logistics.

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 – 100%

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 – 100%.

Major Task 5: Data analysis and interpretation for the Specific Aim 1 results

Milestone 1:

1. Manuscript preparation on the effect of DRP-RF on CBF and cerebrovascular autoregulation 24-36 – 100% (manuscript published)
2. Manuscript preparation on the effect of DRP-RF on tissue hypoxia, neuronal survival and oxidative stress 24-36 – 100% (manuscript published)

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 – 100%

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 – 100%

Subtask 2. Cognitive deficits (Passive avoidance - learning, Y- maze working and Novel Object - recognition memory) – 20-32 months – 100%.

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 – 100%

Subtask 2. Quantitative DTI for apparent diffusion coefficient (ADC), quantitation of edema and contusion volume – 100%

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 – 100%

Subtask 2. Capillary density evaluation by alkaline phosphatase staining – 20-32 months – 100%.

Subtask 3. Inflammation evaluations (microglia and astrocytes activation) – 20-32 months – 100%

Major Tasks 4: Data analysis and interpretation for Specific Aim 2 – 24-36 months – 100%

Milestone 2:

1. Manuscript preparation on effect of DRP-RF on contusion, CBF and behavioral
2. Manuscript preparation on effect of DRP-RF inflammation and cell death

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 period of the project, we have completed all the Tasks that were set in SOW.

I. 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).

1.1. On Fig. 1 is presented a general experimental protocol that, 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 was 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).

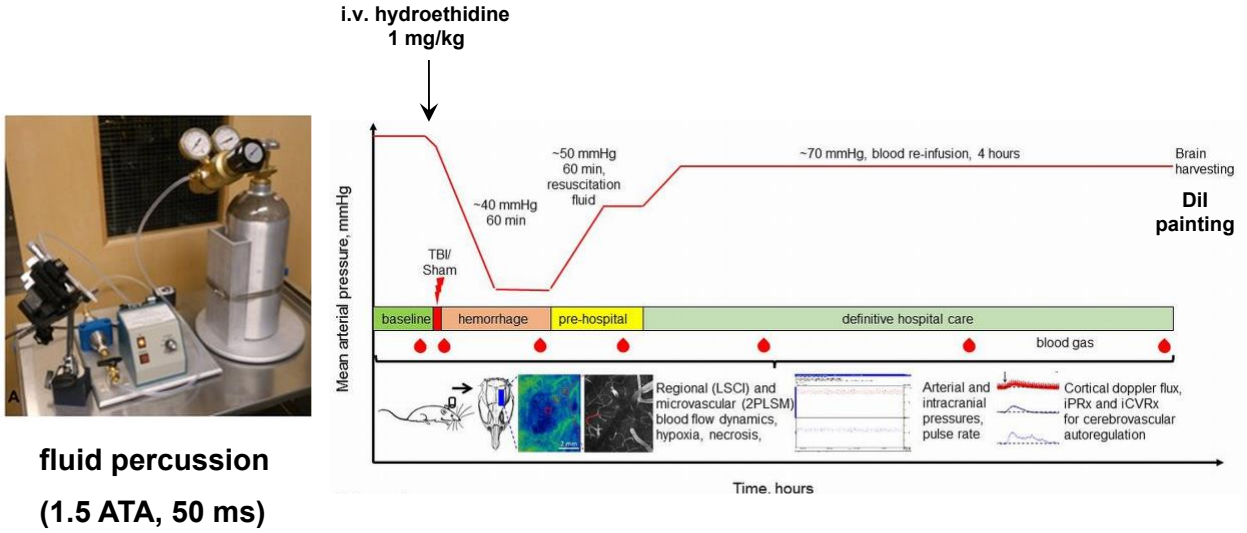

Fig. 1 Experimental Protocol

1.2. 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 the 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. Figure 3. Represent comparative effects of the lactated Ringer, Hetastarch, and hypertonic saline DRP-added RFs on cerebral microcirculation and coagulopathy. Colloid-based DRP-RF was more effective than crystalloid and hypertonic—based DRP-RF.

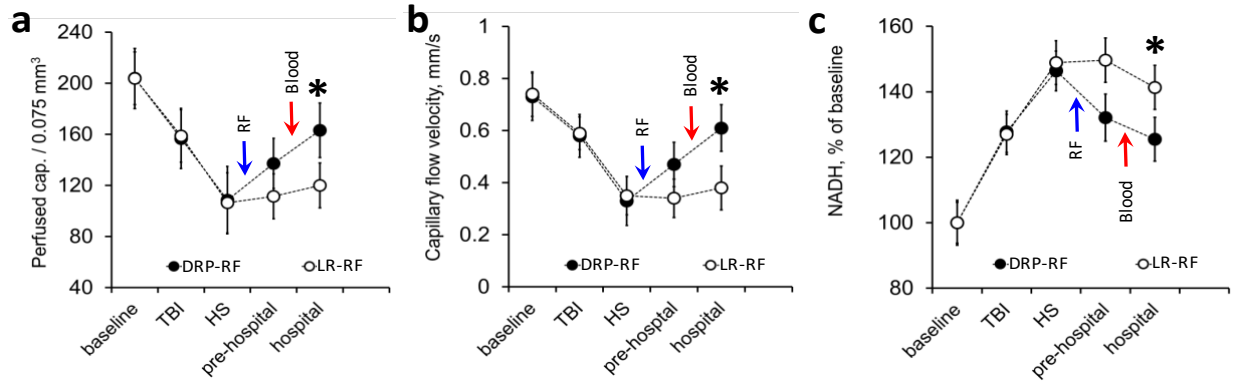

Fig. 2 Time series showing that resuscitation with crystalloid-based DRP-RF improves cerebral microvascular perfusion and tissue oxygenation impaired by TBI/HS, as shown by a) increased number of perfused capillaries; b) increased capillary flow velocity; c) increased tissue oxygenation (NADH decrease).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

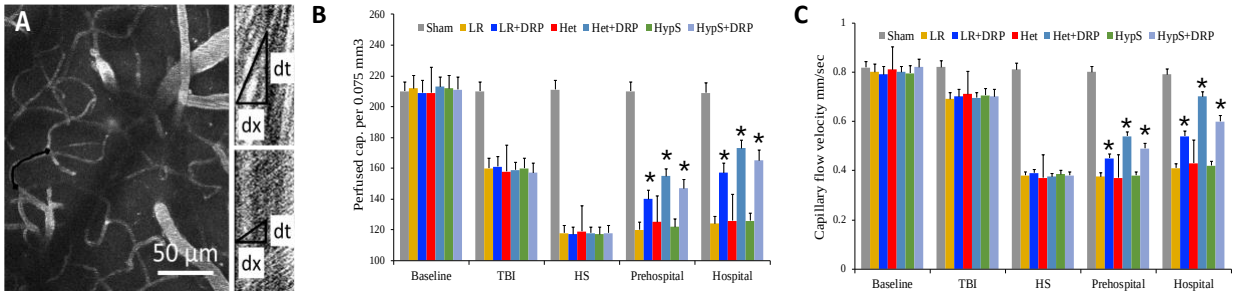

Fig. 3. RF+DRP improves impaired by TBI+HS cerebral microvascular perfusion A. 2PLSM image, representing volume, where capillaries flow velocities were recorded. Top right: Line-scan from the capillary before and after (bottom right) RF+DRP injection showing flow velocity increase. B. Resuscitation with RF+DRP better attenuated capillary microthrombi and re-recruited collapsed after HS capillaries. C. RF+DRP better improved cerebral microvascular perfusion, as it is shown by increased flow velocities.

1.3. 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.

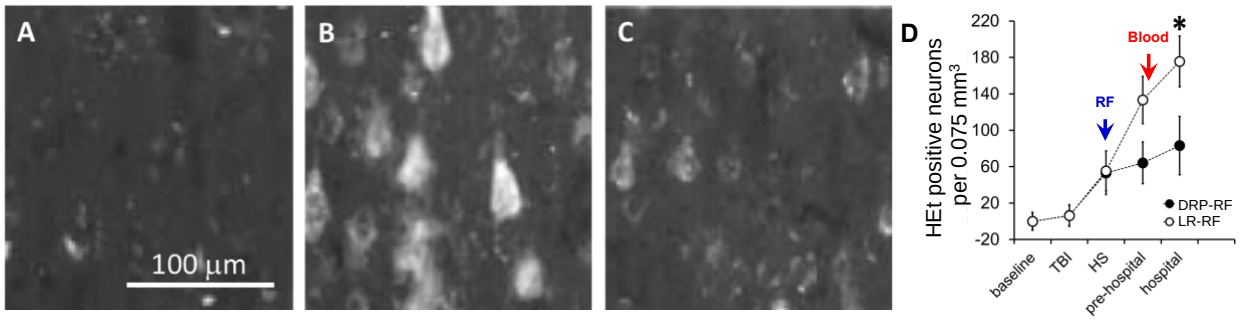

Fig. 3 Resuscitation with DRP-RF reduces superoxide production in cortical neurons after TBI with HS: a) Representative image of a rat cortex at baseline without ET positive neurons; b) Neurons with diffuse cytosolic ET fluorescence in a rat cortex from LR-RF group by the end of the experiment; c) and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

1.4. Using DiI vascular painting technique, we evaluated microvascular changes in the extracted brain and found massive microthrombosis in both contralateral and ipsilateral to trauma hemispheres, which 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 opposed to 6.8 ± 0.4 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.

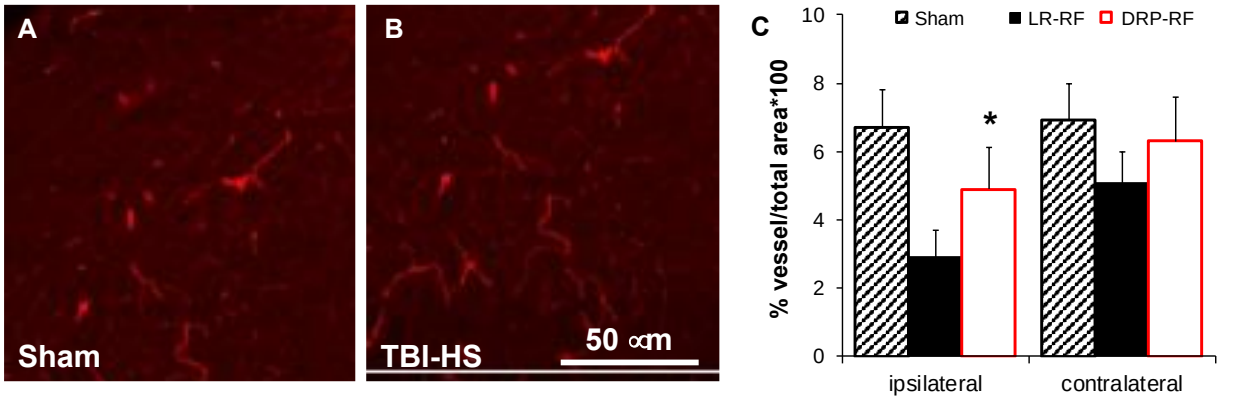

Fig. 4. Resuscitation with DRP-RF reduces microthrombosis in both hemispheres after TBI with HS, as shown by post-mortem DiI vascular painting. a) Cortical microvascular network in Sham mouse brain; and b) after TBI with HS; c) Graph showing reduced cortical microvasculature in LR-RF group and better-preserved 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.

1.5. To evaluate *neuronal survival*, 200 µL of propidium iodide (PI)/saline, which labels only necrotized cells with damaged membranes, was injected intravenously during surgical preparation. CBF and tissue oxygenation reduction after TBI/HS caused progressive necrosis of neurons. DRP-RF reduced the progression of necrosis of neurons, while standard RF (LR) did not decrease the dynamic of necrosis. (Fig. 5). The results are presented in the published manuscript attached. Fig. 6. Represent comparative effects of the lactated Ringer, Hetastarch, and hypertonic saline DRP-added RFs on tissue oxygen supply and neuronal survival.

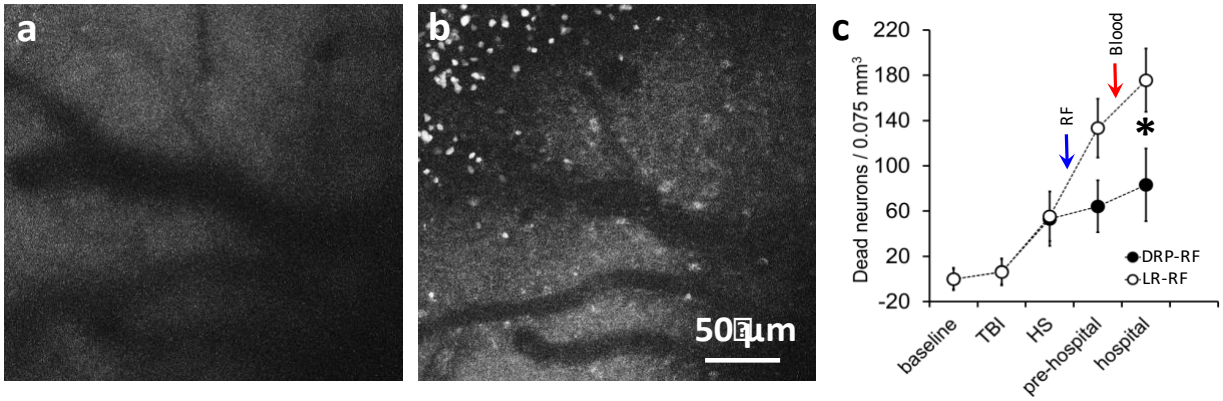

Fig. 5 Resuscitation with DRP-RF is neuroprotective: a) 2PLSM image of a rat cortex at baseline without dead neurons; b) Propidium Iodide stains neurons with damaged membranes reflecting necrosis of neurons after TBI/HS; c) DRP-RF protects neurons from necrosis (*=P<0.05). Mean ± SEM, N=10 rats per group,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

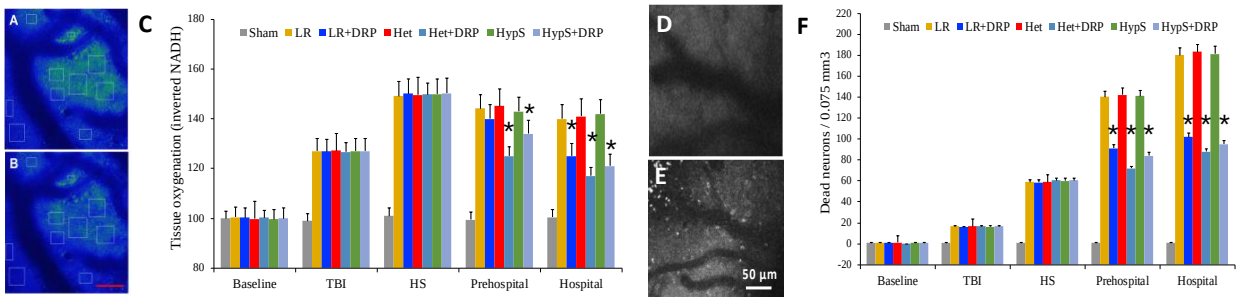

Fig. 6. Improved by RF+DRP microvascular perfusion attenuates tissue hypoxia and protects neurons. A. 2PLSM image of NADH fluorescence in the cortex after TBI+HS and B. after RF+DRP. C. Graph showing better attenuation of brain hypoxia by RF+DRP after TBI+HS. D. 2PLSM image of a rat cortex at a baseline without dead neurons. E. Propidium Iodide stains neurons with damaged membranes reflecting necrosis of neurons after TBI+HS. F. RF+DRP protects neurons from necrosis.

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

II. 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).

2.1. To evaluate behavioral outcomes, we used a battery of sensorimotor and memory, and cognition tests.

Sensorimotor tests included:

Adhesive removal Test for long-lasting sensory and motor deficits, the adhesive tape will be applied to both forepaws, and contact and removal times will be recorded. This test determines whether somatosensory information from the forepaws (tape adhesion) can trigger an instinctive behavioral response (tape removal).

Rotarod performance Test for coordination and motor deficits will be performed using a Rotarod (San Diego Instr., CA) with training and testing sessions before and after TBI/HS. The time to dismount with the increasing speed of rotation will be measured. The test measures the motor dexterity and endurance of animals on the rotating rod, including balance and coordination.

Memory and cognition tests included:

Spatial Alternation in the Y-Maze Test for working memory. Each animal will be placed in the center of the Y-maze and allowed free exploration for five minutes. The total number of arm choices and the number of spontaneous alternations (i.e., where the previous two arm choices differed from the third) will be calculated, computed, and analyzed from the videotaped session.

Novel Object Recognition Test for recognition memory. On day one, rats will be placed in an open arena (60cm x 50cm x 40cm) for a 10-minute habituation. On day two, rats will be placed in

the same arena and exposed to two similar objects for 10 minutes. On day three, rats will be placed in the same arena and exposed to one familiar object (the same objects from day two) and one novel object (distinct in shape and texture) for 10 minutes. A tracking system (Noldus, EthoVision) will be used to calculate the percentage of time spent on object exploration. I.e., the rats will be tested for their interest in a new object as opposed to a familiar object.

Passive Avoidance Test for learning and memory based on classical Pavlovian conditioning. The passive avoidance chamber is partitioned into two sections, one light, and one dark. As the rat moves into the dark section, a mild foot electric shock is delivered through the floor. One day after training, the rat will be placed again into the illuminated section, and the time required for a rat to move into the dark section will be recorded.

Figure 7. represents the neurobehavioral indexes calculated from the behavioral tests battery performed from 1 day to 4 wks after TBI+HS showing better outcomes in RF+DRP groups.

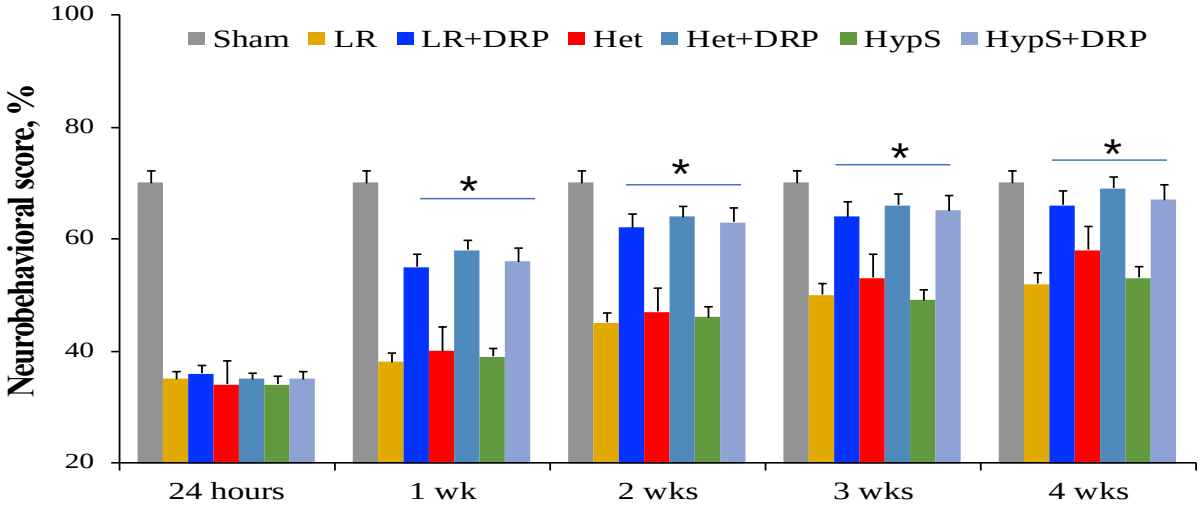

Fig. 7. RF+DRP improves neurological outcome. Neurobehavioral indexes, calculated from behavioral tests battery performed from 1 day to 4 wks after TBI+HS show better improvement in RF+DRP groups ($p<0.05$).

The sensory-motor function was evaluated by rotarod, which revealed that DRP better improves the motor deficit compared to conventional RFs (Fig 8.)

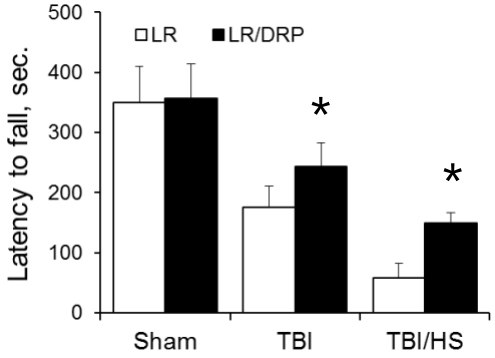

Fig. 8. DRP better improves sensory-motor function as the latency to fall, evaluated by the rotarod test, is significantly longer in DRP groups than in controls (LR) at 1 week after the insult.

Long-lasting sensory and motor deficits were also evaluated by Adhesive Tape Removal. Adhesive tape applied to both forepaws, and contact and removal times were recorded. This test determines whether somatosensory information from the forepaws (tape adhesion) can trigger an instinctive behavioral response (tape removal) (Fig. 9). Two adhesive tapes (0.4 cm2) were applied with equal pressure on each animal paw. The order of placement of the adhesive (right or left) was alternated between each animal and each session. The rat was placed in a transparent plastic box, and the times to contact and remove each adhesive tape were collected, with a maximum of 120 s. Rats were trained daily before (5 d before TBI+HS). Data are presented in Figure above. As expected, the time to remove the adhesive tape was similar in all groups at the baseline. At 24 hrs after TBI+HS, significant differences became apparent between the groups. Both contact and removal times were significantly longer in the TBI+HS group than in the Sham group ($P < 0.01$). Rats n the groups with DRP better performed than rats from LR and Het groups only ($p < 0.05$). By the end of 3 weeks, LR+DRP and Het+DRP rats recovered better than rats resuscitated with LR and Het only.

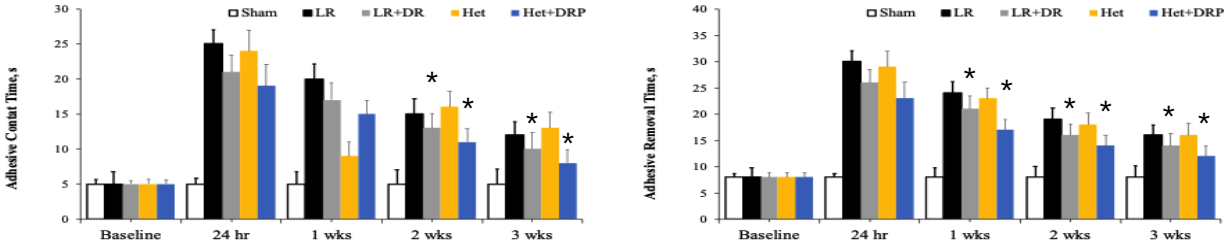

Fig. 9 RF+DRP improves functional recovery. A, B, Bilateral asymmetry/adhesive removal test. Two parameters were monitored for each paw: adhesive tape contact time (A) and adhesive removal time. N= 10/group, mean ± SD.

We evaluated memory and learning by the Novel Object recognition test. Testing occurs in a square maze with two objects presented to the animal. After the learning phase, the animal removed to the cage and one of the objects is replaced by new one. The rodent is then placed back to the maze, so the intact animal should show a tendency to explore the new object for a longer time. The obtained results show that DRP+RF better improves learning and memory than conventional RFs (Fig. 10).

We tested learning and memory by Passive Avoidance Test that is based on classical Pavlovian conditioning. The passive avoidance chamber was partitioned into two sections, one illuminated and one dark. As the rat moved into the dark section, a mild foot electric shock was delivered through the floor. One day after training, a rat was placed again into the illuminated section, and the time required for a rat to move into the dark section was recorded. As expected, the retention time was similar in all groups at the baseline. At 24 hrs after TBI+HS, significant differences became apparent between the groups. Retention latency times were significantly shorter in the TBI+HS group than in the Sham group ($P < 0.01$) reflecting impaired memory and learning. Rats n the groups with DRP better performed than rats from LR and Het groups only ($p < 0.05$). By the end of 4 weeks, LR+DRP and Het+DRP rats recovered better than rats resuscitated with LR and Het only (Fig. 10).

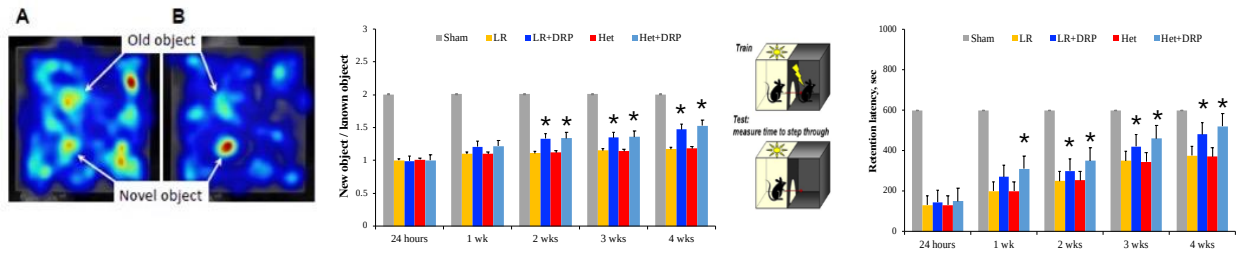

Fig. 10. RF+DRP improves cognitive outcome. DRP improves learning and memory as measured by Novel Object Recognition Test: a) Traumatized rat investigates both, novel and old object, reflecting impaired memory; b) Intact rat investigates mainly novel object as it remembers the old one; c) Graph shows better preserved/recovered memory dynamics in DRP group. d) Passive Avoidance Test for learning and memory assessment. e) Graph showing better improved learning and memory in DRP+RF groups.

We also evaluated spatial working memory by Y-maze. Testing occurs in a Y-shaped maze with three plastic arms oriented in a 120° angle. One arm of the Y-maze is blocked and the animal is allowed to explore the other two arms. The rodent is then placed in the start arm and the blocked arm is opened, so the animal should show a tendency to enter the formerly blocked arm more frequently. DRP+RF better improves spatial working memory than conventional RFs (Fig. 11).

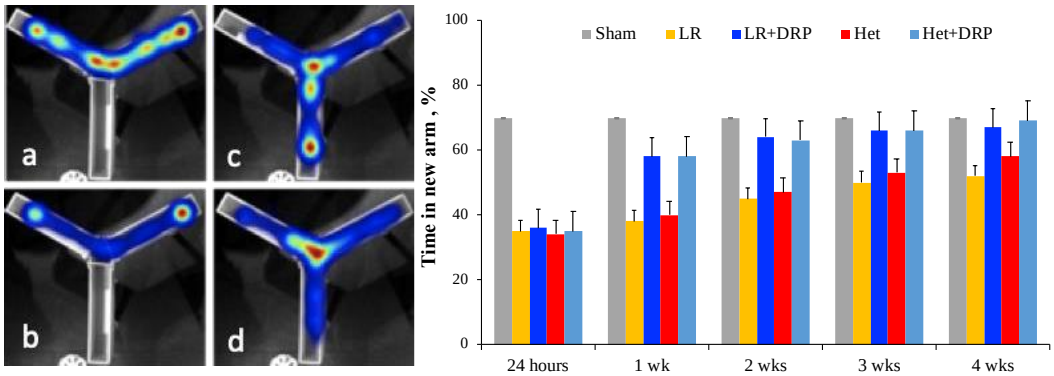

Figure 11. DRP improves spatial working memory as measured by Y-maze: a) Intact rat actively investigate the open two hand of the Y-maze; b) traumatized rat is not active; c) after opening of the third hand of the Y-maze, intact rat actively investigates the new hand of the maze; d) due to deficiency 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 dynamics in DRP group. N= 10/group, mean ± SD.

2.2. Using MRI we compared the effects of DRP+RF vs. RF on anatomical and functional outcome

Using ASL MRI, we demonstrated that DRP-addition improves global cerebral blood flow after TBI+HS, confirming our 2PLSM data (Fig. 12).

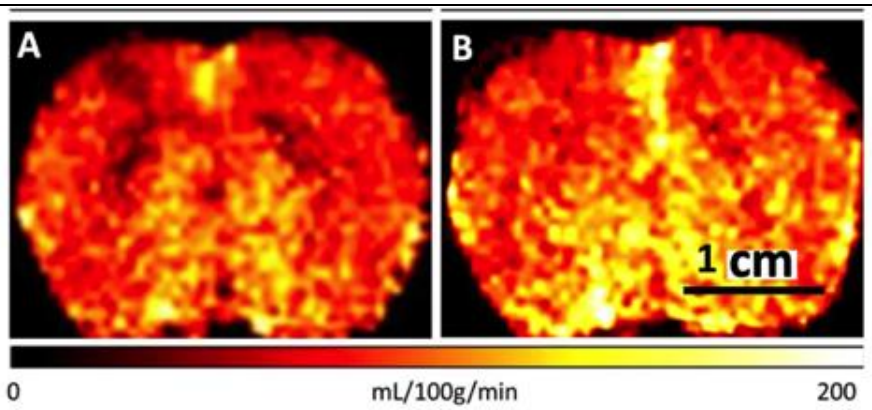

Fig. 12 RF+DRP improves global CBF A. Low global CBF after resuscitation with RF. B. Global CBF is higher after resuscitation with RF+DRP.

In addition, a hypercapnia test during MRI revealed better presented cerebral vascular reactivity (CVRx) in animals resuscitated with RF+DRP compared to conventional RFs (Fig. 13.).

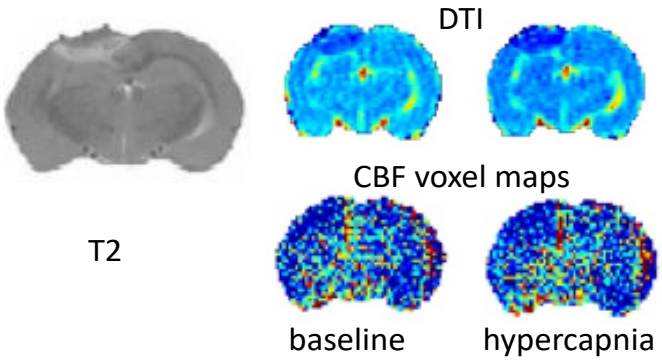

Fig. 13 Representative MRI images showing cerebral vascular reactivity (CVRx) changes after TBI/HS measured by MRI and hypercapnia test. Resuscitation with DRP better preserved CVRx.

2.3. Using histochemical and immunohistochemical analysis, we have confirmed our in-vivo results that DRP addition has a neuroprotective and anti-inflammatory effect (Fig. 14).

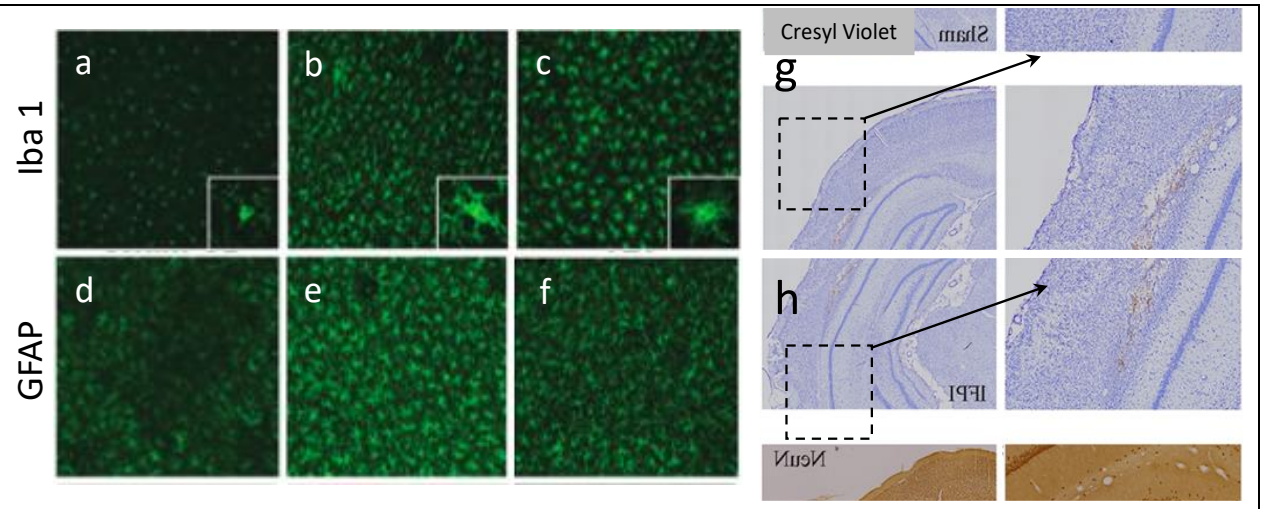

Fig 14. Rat brain sections stained for Iba 1 (microglia) and GFAP (reactive astrocytes) showing that TBI+HS induce reactive gliosis **(b and e)** compared to sham **(a and d)**. DRP addition to RF reduces inflammation and glial reactivity **(c and f)**. Nissl micrographs of sham **(g)** and TBI brain section with the impact site.

Taken together, we have shown that DRP-added Hetastarch was more efficient than DRP-added Hypertonic Saline, which was, in turn, more efficient than DRP-added Lactated Ringer. Rheological modulation of blood flow using RF+DRP effectively restores cerebral microcirculation, reduces hypoxia, microthrombosis formation, and mitochondrial oxidative stress, protects neurons and improves neurologic outcome after TBI+HS compared to conventional volume expansion with RF. In addition, RF+DRP requires an infusion of a smaller volume to improve tissue perfusion and oxygen utilization which reduces brain edema formation due to hypervolemia, which often occurs with standard fluid resuscitation.

The Pittsburgh sub-awardee competed 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.

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 by the pump which prohibited recording accurate data collection on storage degradation.

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 turbulent flow system.

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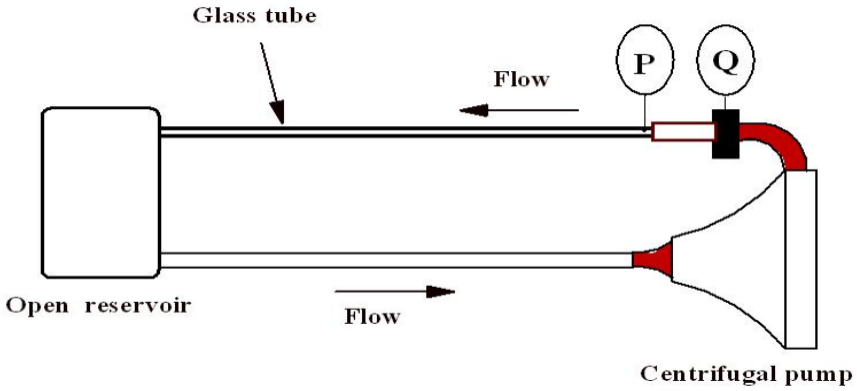


Fig. 1

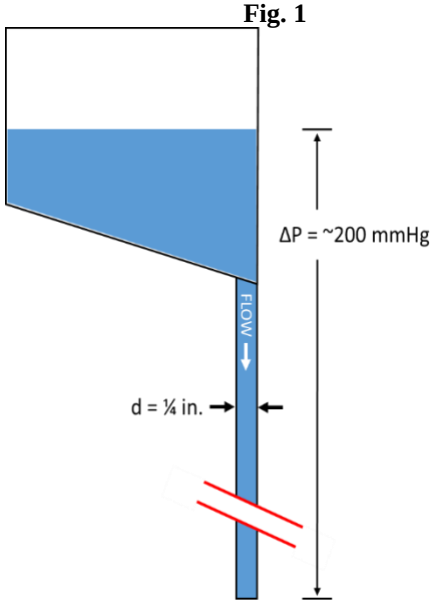


Figure 2

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.

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:

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

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	

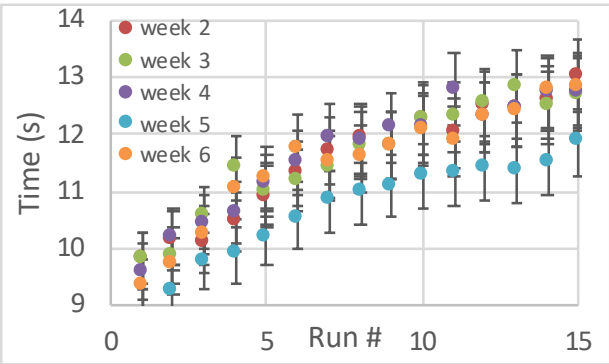


Figure 3: Effect of number of runs on time length of run the samples prepared from 4000 ppm PEO solution

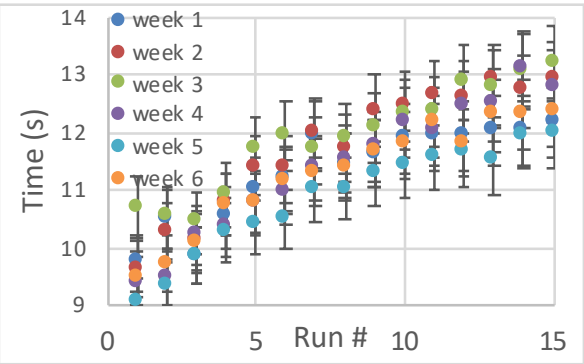


Figure 4: Effect of number of runs on time length of run the samples prepared from 1000 ppm PEO solution

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.

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 were measured weekly for DRP viscosity and elasticity as well as drag reducing ability (see below) to ensure sample viability.

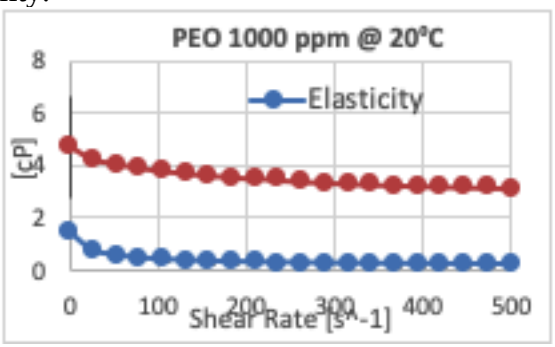


Figure 5: Viscosity and elasticity of the PEO-1000 solution recorded by Vilastic-3 viscoelasticity analyzer

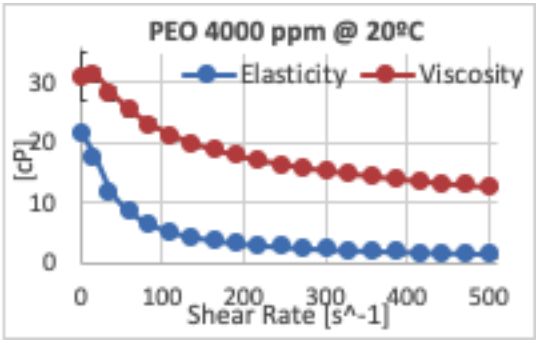
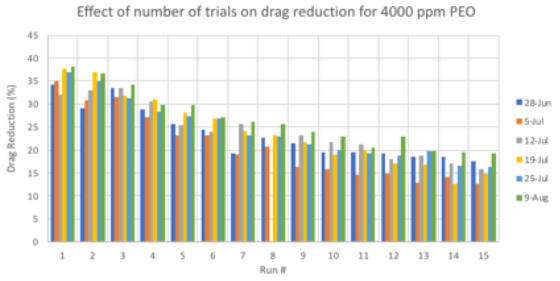
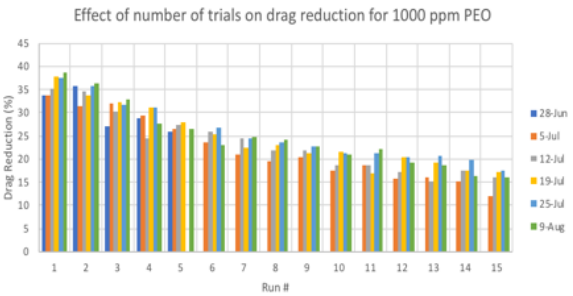


Figure 6: Viscosity and elasticity of the PEO-4000 solution recorded by Vilastic-3 viscoelasticity analyzer

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.





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.

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.

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.

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.

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

Vilastic Viscoelasticity Analyzer

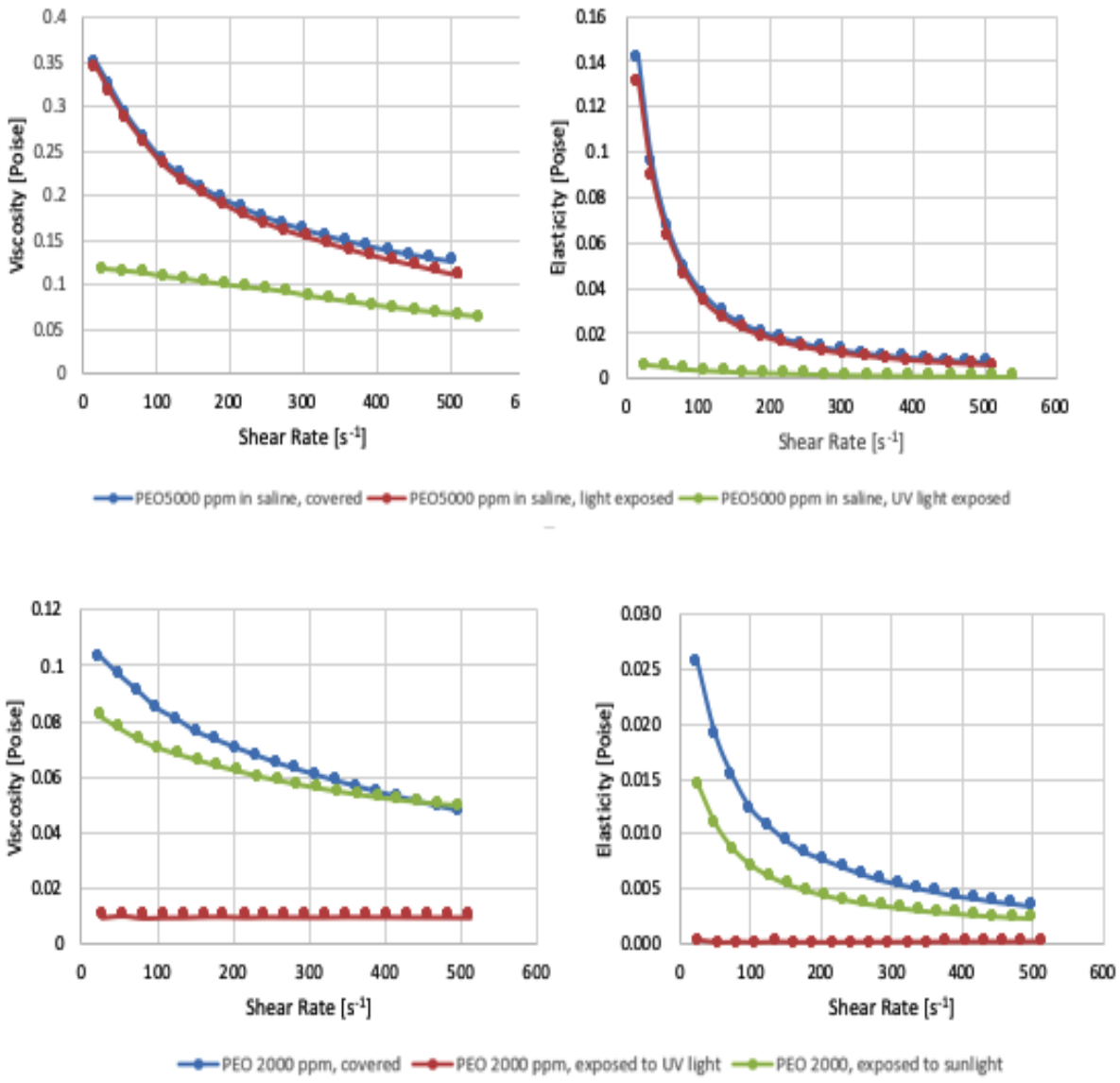


Figure 7*. Viscosity and elasticity of PEO 5000 ppm solutions after exposure to no light (blue), grow light (red), and UV light (green) (TOP). Viscosity and elasticity of PEO 2000 ppm solutions after exposure to no light (blue), sunlight (green), and UV light (red) (BOTTOM).*

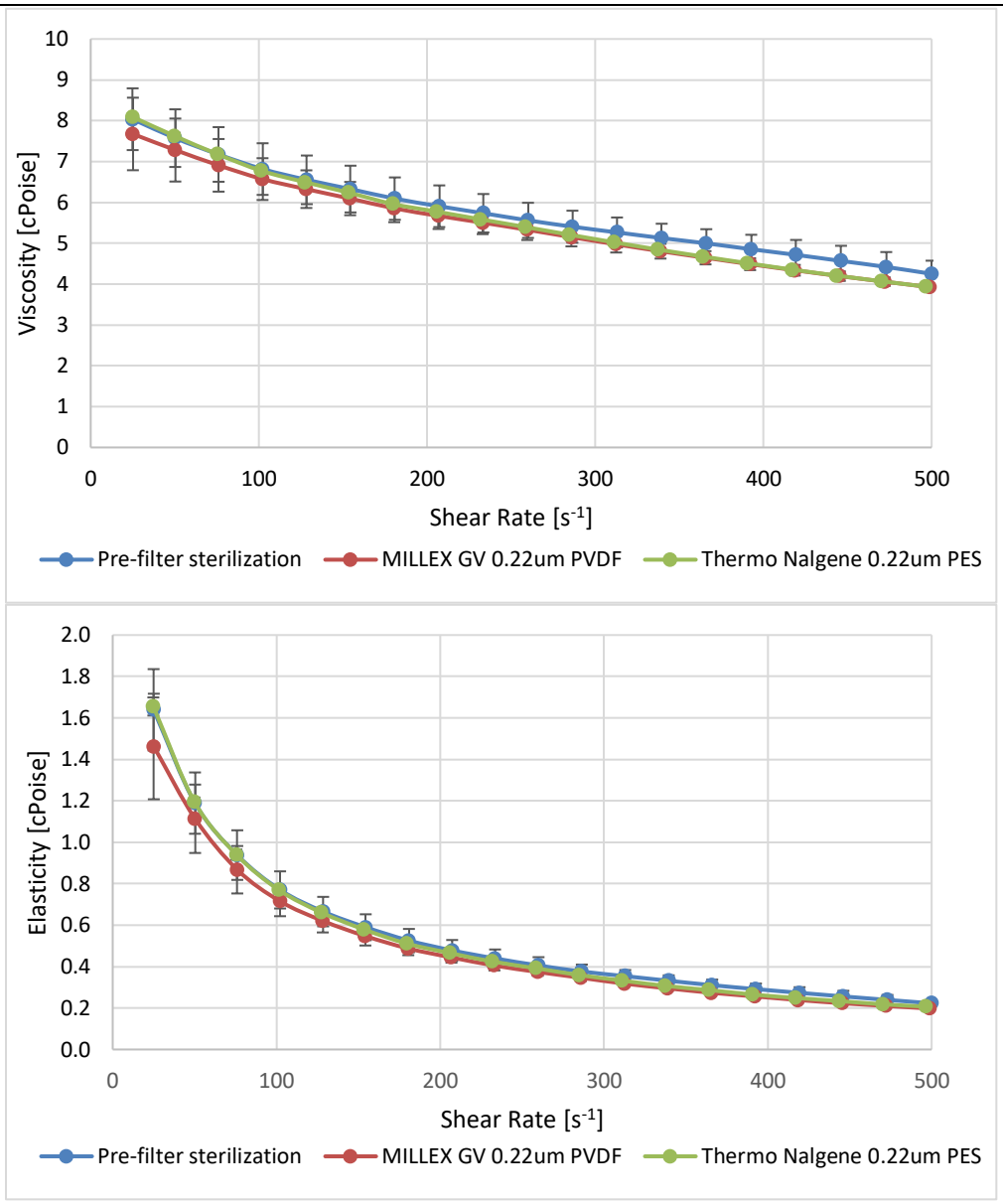


***Figure** 8. Viscosity (top) and elasticity (bottom) of 2000 ppm PEO solutions following sterilization of 0.22 filter membranes.*

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. We developed a strong protocol for producing the primary concentrated PEO solution and its storage, from which DRP-RF is prepared and used without delay and with no degradation of the polymer molecules in solutions.

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:

- 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, the 46th Annual Meeting of the International Society for Oxygen Transport to Tissue, July 1-5, 2018, Seoul, S. Korea
- 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, Joint Symposium of the International and National Neurotrauma Societies and AANS/CNS Section on Neurotrauma and Critical Care, August.11-16, 2018, Toronto, Canada.
- 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, Neurocritical Care Society's Annual Meeting 2018, September 25-28, Boca Raton, FL.
- 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, Military Health System Research Symposium, Kissimmee, FL, August 20-23, 2018
- 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, International Stroke Conference, Honolulu, HI, February 06-02, 2019
- D. Bragin, D, Lara, O. Bragina, M. Kameneva, E. Nemoto. Neuroprotective Resuscitation Fluid for TBI with HS, UNM HSC 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, National Neurotrauma Symposium, Pittsburgh, PA, June.29 - July03, 2019.
- 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, the 29th International Symposium on Cerebral Blood Flow, Metabolism and Function, July 4 – 7, 2019, Yokohama, Japan.
- 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. the 47th Annual Meeting of the International Society for Oxygen Transport to Tissue, July 27-31, 2019, Albuquerque, NM, USA
- Denis E Bragin, Olga A Bragina, Lusy Berliba, M.V. Kameneva, and 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. Presented at the scientific breakout session Military Health System Research Symposium, August 19-22, 2019б Kissimmee, FL
- Denis E Bragin, Olga A Bragina, Lucy Berliba, Marina V Kameneva, Edwin M Nemoto. Improved Cerebral Perfusion Pressure and Microcirculation by Drag Reducing Polymer-Enforced Resuscitation Fluid after TBI and Hemorrhagic Shock. International Symposium on Intracranial Pressure and Neuromonitoring, September.08-11, 2019-ICP, Lewen, Belgium.
- Denis E. Bragin, Olga A. Bragina, Marina V. Kameneva, Edwin M. Nemoto, and Afshin A. Divani. The Addition of Drag Reducing Polymers to resuscitation fluids provides neuroprotective properties in Brain Injury, Brain & Brain PET 2022, the 30th International Symposium on Cerebral Blood Flow, Metabolism and Function, Glasgow UK, 29 May - 1 June 2022.
- Denis E Bragin, Olga A Bragina, Afshin Divani, Marina V Kameneva, Edwin M Nemoto. Enhanced by Drag Reducing Polymer Resuscitation Fluid Improves Cerebral Microcirculation, Tissue Oxygenation, Neuronal Survival and Neurologic Outcome after Traumatic Brain Injury with Hemorrhagic Shock, Military Health System Research Symposium, Kissimmee, FL from 12-15 September 2022.
- Bragin DE, Bragina OA, Kameneva MV, Nemoto EM, Divani AA. Drag Reducing Polymers Added to Resuscitation Fluids Provides Neuroprotection in a Rat Model of Brain Injury. The 3rd European Association of Neurosurgical Societies Trauma and Critical Care Update Meeting, Leuven, Belgium, Sep 12-14, 2022.
- Denis E. Bragin, Olga A. Bragina, Marina V. Kameneva, Edwin M. Nemoto, and Afshin A. Divani, Drag-Reducing Polymers Transform any Resuscitation Fluid Into Neuroprotective, Society for Critical Care Medicine 2023, Critical Care Congress, San Francisco, CA, January 21-24, 2023
- Denis E Bragin, Olga Bragina, Marina Kameneva, Afshin A Divani, Edwin M Nemoto, Sex-specific And Dose-dependent Effects Of Drag-reducing Polymers On Post-traumatic Ischemia In Rats After Traumatic Brain Injury Of Different Severity, International Stroke Conference, Dallas, TX, February 8-10, 2023.

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.

Nothing to Report

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).

We have successfully completed the project. As a result of the effort we already published 4 manuscripts, 1 is in preparation and another 2 are planned to be prepared in the year 2023. The results of the study were presented as oral and poster presentations at 16 national and international conferences.

We have developed a detailed transition plan to get the product to clinical use. The product has been already successfully tested on a rat model of TBI+HS through DOD funding (W81XWH-17-2-0053, PI: Bragin). During this project, we demonstrated a neuroprotective efficiency of DRP via reduction of cerebral ischemia, microthrombosis and mitochondrial oxidative stress. Thus, before IND and clinical trial initiation, the next necessary step is to test the product on a large animal model of TBI+HS, which is proposed in this application. The addition of a gyrencephalic animal polytrauma model both capitalizes on this work and greatly enhances the end-product. Developing a therapy that prolonged survivability would be of huge benefit, especially if there was a chance for neuroprotective effects. Following are the required elements of the transition plan.

A novel advanced resuscitation fluid (6% Hetastarch in Lactated Electrolyte Injection (Hextend) with the addition of Drag Reducing Polymers (Hex; DRP-Hex) is the primary "potential product" that is being tested for efficacy in the combined swine model of traumatic brain injury and hemorrhagic shock (TBI+HS) that we propose in follow-up current applications.

We have already submitted the following applications to test DRP+RF on a swine model of TBI+HS:

04.20.2020 – JW200235 A Novel Advanced Resuscitation Fluid for a Swine Model of Hemorrhagic Shock with Traumatic Brain Injury. – Excellent score but not supported.

05.01.2020 – PR201812 A Novel Advanced Resuscitation Fluid for a Swine Model of Hemorrhagic Shock with Traumatic Brain Injury. – Outstanding score but not supported.

06.23.2021 – JW210477 A Novel Advanced Resuscitation Fluid for a Swine Model of Hemorrhagic Shock with Traumatic Brain Injury. – Excellent score but not supported.

05.16.2022 – JW220138 A Novel Advanced Resuscitation Fluid for a Swine Polytrauma Model (Traumatic Brain Injury and Hemorrhagic Shock) – Excellent score but not supported.

We continue our effort to secure funding and plan to submit NIH (R61/R33) and DOD applications this year.

Intellectual Property: U.S. Patent was issued for D.E. Bragin, E.M. Nemoto & M.V. Kameneva in conjunction with STC.UNM, the Technology Transfer office, on 09/19/2017 (Bragin, D.E., Nemoto, E.M., & Kameneva, M.V. (2017). U.S. Patent No. US9763975B1 "Rheologic Treatment of Brain Ischemia by Drag Reducing Polymers." Washington, DC: U.S. Patent and Trademark Office).

Commercialization Strategy: The ultimate deliverable would require FDA approval. Based on the patent (US Patent 9,763,975 B1, Sept. 19, 2017) owned by Drs. Bragin, Nemoto, and Kameneva on the use of DRP, a startup company Shearit, LLC was formed (https://www.shearitllc.com) . Drs. Bragina and Kameneva are Scientific Advisers in the company.

ShearIT intends to develop DRP as a virtual company, using qualified vendors to perform technical services under contract. ShearIT's Chief Technical Officer founded and managed a large Contract Development and Manufacturing Company and has significant experience in this arena. The goal is to demonstrate clinical efficacy during phase 2 clinical trials, then sell the IP and data package to a pharmaceutical company. In order to enter into an IND and phase one human clinical trials, both active pharmaceutical ingredient (API) and sterile injection dosage form will need to be manufactured in full compliance with cGMP. That will start with development of a cGMP synthetic process. DavosPharma-Seqens has been selected to perform this work at the companies California facility. They will develop the synthetic process and prepare small-scale development and scale-up batches of the API, defining the specifications and molecular weight distribution. This material will be used for dosage form development and continuing animal studies. The goal for molecular weight distribution of the 4000 kDa API will likely be 2000 to 5000 kDa. DavosPharma-Seqens can manufacture cGMP material at their Boston facility and will be responsible for development and validation of analytical methods for the API, as well as API stability assessment.

ShearIT has not yet selected a contract manufacturer of the sterile injection product but is evaluating possible partners. A vendor capable of commercial product of the product will be selected. Clinically, the product will most likely be by piggy back infused i.v. in normal saline for patient administration. The vendor will develop the dosage form, e.g. a 10-mL vial presentation, develop and validate analytical methods and sterility procedures, as well as assess stability of the dosage form. The Chief Technical Officer of ShearIT, as well as the Quality Assurance function will work closely with the vendor to assure a quality product is developed for human clinical trials. Then as clinical trials progress, batch sizes will be scaled-up and sterility processes evaluated.

Translation and Implementation: Upon completion, we will bring a novel, highly efficient in small-volume resuscitation fluid for TBI/HS that can be easily administered by first responders or combat medics in the unique conditions of the battlefield and/or severe civilian trauma. As the next step, we will conduct Good Laboratory Practices (GLP) studies at LBRI to demonstrate safety and efficacy in preclinical studies using swine to show that DRP solutions infused in these animals are safe over long periods while monitoring all vital organ functions. The obtained results will be used for the initiation of clinical research related to resuscitation and neuroprotection after TBI in patients with hemorrhagic shock. T hese studies would provide the basis for the application of an IND to conduct clinical trials in patients to show safety and efficacy.

Market size, potential, cost of research and development The *market size* is worldwide distribution in both military and civilian applications. This distribution is not limited to TBI, but may also include any severe trauma, as well as any shock condition including sepsis, pneumonia resulting in systemic arterial hypotension, microvascular shunting and systemic metabolic acidosis. For sepsis alone, it has been estimated to occur in 150 million cases each year worldwide. In the US, there are ~2 million TBI each year and over 10 million worldwide. The market potential is obviously very large. Even at $20/unit with the USA using approximately 10 million transfusion fluid bags per year for all clinical applications, the market potential would be about $200M dollars/year, which could be a gross underestimate.

The *cost of research and development* to bring the DRP resuscitation fluids to market is estimated to require $3-4 M for Good Laboratory Practices (GLP) studies evaluating the effects of the resuscitation fluid to obtain an Investigational New Drug application (IND), which could be funded by the NIH without endangering intellectual property issues. The research costs to bring the product to the clinic will require clinical trials in various clinical conditions including TBI, HS, sepsis, stroke, etc. which is estimated to cost $5-$10M. This would have to be raised from the private sector including angel investors. The *potential use of this product* as previously mentioned is very large if/when proved effective in treating many clinical conditions attributable to low microvascular perfusion.

Time table.

1. April 5, 2023 Phase I SBIR NINDS application of DRP in traumatic brain injury.
2. July 1, 2023, start Phase I SBIR NINDS study for DRP in traumatic brain injury.
3. September 5, 2024, apply for Phase II SBIR for DRP in traumatic brain injury.
    3.a DavosPharma-Seqens: Sterile GMP production of the 2000-4000kDA DRP.
    3.b. Phase II In-Life studies (Lovelace Biomedical) in rats (toxicology studies) and in minipigs defining the maximum functional dose and tissue deposition and pharmacokinetics (PK) and pharmacodynamic (PD) studies with the consultation of a toxicologist consultant (Stephen Montgomery).
    3.c. Bioanalytic laboratory (Worldwide) for sample analysis.
    3.d. Planning information for Clinical safety studies for a pre-IND meeting.
    3.e. Obtain IND and perform a Clinical Safety study.
4. Seek Pharmaceutical companies (Pfizer, Astra Zeneca, Bristol Meyer Squibb, etc.) to partner in supporting Phase I-III Clinical trials.

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

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

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.

No significant changes

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
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. PMID: 30178321; PMCID: PMC6314472.

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, Bragina OA, Trofimov A, Berliba L, Kameneva MV, Nemoto EM. Improved Cerebral Perfusion Pressure and Microcirculation by Drag Reducing Polymer-Enforced Resuscitation Fluid After Traumatic Brain Injury and Hemorrhagic Shock. Acta Neurochir Suppl. 2021;131:289-293. doi: 10.1007/978-3-030-59436-7_54. PMID: 33839860; PMCID: PMC8086029.

Bragin DE, Bragina OA, Berliba L, Kameneva MV, Nemoto EM. Addition of Drag-Reducing Polymers to Colloid Resuscitation Fluid Enhances Cerebral Microcirculation and Tissue Oxygenation After Traumatic Brain Injury Complicated by Hemorrhagic Shock. Adv Exp Med Biol. 2021;1269:283-288. doi: 10.1007/978-3-030-48238-1_45. PMID: 33966231.

The manuscript comparing colloid based vs. crystalloid vs. hypertonic saline DRP-added resuscitation fluid is in preparation to be submitted to a Journal of Trauma and Acute Care Surgery.

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).*

- 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.
- 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.
- 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, 2018, A-136.

- 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.
- 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.
- 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.
- 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, 2019, 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, 2019, P. 93.
- D.E. Bragin, O.A. Bragina, L. Berliba, M.V. Kameneva and E.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. Abstract fo the Military Health System Research Symposium 2019.
- Denis E Bragin, Olga A Bragina, Lucy Berliba, Marina V Kameneva, Edwin 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, 2019.
- Bragin DE, Bragina OA, Kameneva MV, Nemoto EM, Divani AA. The Addition of Drag Reducing Polymers to resuscitation fluids provides neuroprotective properties in Brain Injury. *JCBFM BRAIN and BRAIN-PET 2022 abstracts*, 42(1S) P:90-91.

- Denis E Bragin, Olga A Bragina, Afshin Divani, Marina V Kameneva, Edwin M Nemoto. Enhanced by Drag Reducing Polymer Resuscitation Fluid Improves Cerebral Microcirculation, Tissue Oxygenation, Neuronal Survival and Neurologic Outcome after Traumatic Brain Injury with Hemorrhagic Shock, online Abstracts for Military Health System Research Symposium, Kissimmee, FL from 12-15 September 2022.
- Bragin DE, Bragina OA, Kameneva MV, Nemoto EM, Divani AA. Drag Reducing Polymers Added to Resuscitation Fluids Provides Neuroprotection in a Rat Model of Brain Injury. Online abstracts for the 3rd European Association of Neurosurgical Societies Trauma and Critical Care Update Meeting, Leuven, Belgium, Sep 12-14, 2022.
- Bragin, Denis; Bragina, Olga; Kameneva, Marina; Nemoto, Edwin; Divani, Afshin. 508: rag-reducing polymers transform any resuscitation fluids into neuroprotective. Critical Care Medicine 51(1): p 241, January 2023. | DOI: 10.1097/01.ccm.0000907760.18325.aa

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.*

- 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, the 46th Annual Meeting of the International Society for Oxygen Transport to Tissue, July 1-5, 2018, Seoul, S. Korea, Oral Presentation.
- 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, Joint Symposium of the International and National Neurotrauma Societies and AANS/CNS Section on Neurotrauma and Critical Care, August.11-16, 2018, Toronto, Canada. Poster.
- 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, Neurocritical Care Society's Annual Meeting 2018, September 25-28, Boca Raton, FL., Oral Presentation.
- 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, Military Health System Research Symposium, Kissimmee, FL, August 20-23, 2018, Oral Presentation
- 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, International Stroke Conference, Honolulu, HI, February 06-02, 2019, Poster.
- D. Bragin, D, Lara, O. Bragina, M. Kameneva, E. Nemoto. Neuroprotective Resuscitation Fluid for TBI with HS, UNM HSC Neuroscience Day, Albuquerque, NM, 03/17/2019, Poster.

- 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, National Neurotrauma Symposium, Pittsburgh, PA, June.29 - July03, 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, 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. 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, and 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. Presented at the scientific breakout session Military Health System Research Symposium, August 19-22, 2019б Kissimmee, FL, Oral Presentation.
- Denis E Bragin, Olga A Bragina, Lucy Berliba, Marina V Kameneva, Edwin M Nemoto. Improved Cerebral Perfusion Pressure and Microcirculation by Drag Reducing Polymer-Enforced Resuscitation Fluid after TBI and Hemorrhagic Shock. International Symposium on Intracranial Pressure and Neuromonitoring, September.08-11, 2019-ICP, Lewen, Belgium, Poster
- Denis E. Bragin, Olga A. Bragina, Marina V. Kameneva, Edwin M. Nemoto, and Afshin A. Divani. The Addition of Drag Reducing Polymers to resuscitation fluids provides neuroprotective properties in Brain Injury, Brain & Brain PET 2022, the 30th International Symposium on Cerebral Blood Flow, Metabolism and Function, Glasgow UK, 29 May - 1 June 2022, Flash Oral Presentation.
- Denis E Bragin, Olga A Bragina, Afshin Divani, Marina V Kameneva, Edwin M Nemoto. Enhanced by Drag Reducing Polymer Resuscitation Fluid Improves Cerebral Microcirculation, Tissue Oxygenation, Neuronal Survival and Neurologic Outcome after Traumatic Brain Injury with Hemorrhagic Shock, Military Health System Research Symposium, Kissimmee, FL from 12-15 September 2022, Poster.
- Bragin DE, Bragina OA, Kameneva MV, Nemoto EM, Divani AA. Drag Reducing Polymers Added to Resuscitation Fluids Provides Neuroprotection in a Rat Model of Brain Injury. The 3rd European Association of Neurosurgical Societies Trauma and Critical Care Update Meeting, Leuven, Belgium, Sep 12-14, 2022, Poster.
- Denis E. Bragin, Olga A. Bragina, Marina V. Kameneva, Edwin M. Nemoto, and Afshin A. Divani, Drag-Reducing Polymers Transform any Resuscitation Fluid Into Neuroprotective, Society for Critical Care Medicine 2023, Critical Care Congress, San Francisco, CA, January 21-24, 2023, Flash Oral Presentation.
- Denis E Bragin, Olga Bragina, Marina Kameneva, Afshin A Divani, Edwin M Nemoto, Sex-specific And Dose-dependent Effects Of Drag-reducing Polymers On Post-traumatic Ischemia In Rats After Traumatic Brain Injury Of Different Severity, International Stroke Conference, Dallas, TX, February 8-10, 2023, Poster.

- **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.

  D.E. Bragin, E.M. Nemoto & M.V. Kameneva, "Novel Hemorheologic Approach for the Treatment of Brain Ischemia by Drag Reducing Polymers", US Patent #14/789,669, July 01 2017.

- **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:	*Afshin Divani*
Project Role:	*PI*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*3 (10% funded)*
Contribution to Project:	*Overseen the project.*

Sub-award

Name:	*Denis Bragin*
Project Role:	*Sub-award PI*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*1.5 (34.5% funded)*
Contribution to Project:	*Dr. Bragin performed data interpretation and new pre-application preparation. Pre-application submission*

Name:	*Olga Bragina*
Project Role:	*Associate Research Scientist*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*1.5 (75% funded)*
Contribution to Project:	*Has performed data analysis and interpretation.*

Name:	*Lucy Berliba*
Project Role:	*Research Associate*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*1.5 (75% funded)*
Contribution to Project:	*Has performed data analysis and interpretation.*

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.

N/A

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: Divani A. **Org:** Dept. of Neurology, University of New Mexico School of Medicine/ Lovelace Biomedical Research Institute (Sub-contractor)

Award Amount: $1,416,397.00

Study/Product Aim(s)

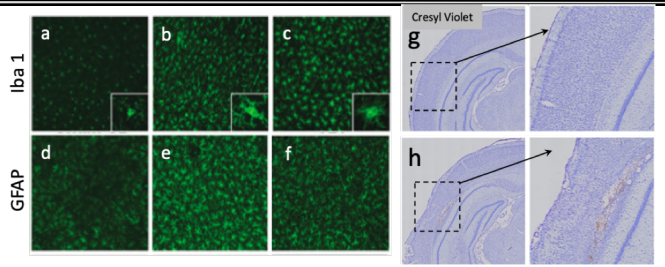
Current resuscitation fluids for traumatic brain injury with hemorrhagic shock (TBI/HS) do not ameliorate impaired cerebral microvascular flow leading to hypoxia, neuronal death, increased mortality and poor neurological outcome. Nanomolar concentrations of blood soluble intravenous drag reducing polymers (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.



Rat brain sections stained for Iba 1 (microglia) and GFAP (reactive astrocytes) showing that TBI+HS induce reactive gliosis (b and e) compared to sham (a and d). DRP addition to RF reduces inflammation and glial reactivity (c and f). Nissl micrographs of sham (g) and TBI brain section with the impact site.

Obtained results will be used for the preproposal "A Novel Advanced Resuscitation Fluid for Swine Model of Traumatic Brain Injury with Hemorrhagic Shock" submitted to DOD and NIH.

Timeline and Cost

Activities CY	17	18	19	21	22
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**	**$300K**	**$250K**

Updated: 01/12/2023

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

- N/A

Budget Expenditure to Date

Projected Expenditure: $1,416K

Actual Expenditure: $1,416K