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13. SUPPLEMENTARY NOTES

AA. ABSARACA

The goal of this project is to test the therapeutic potential of freeze dried platelets-FDPlts (Thrombosomes)- in disease conditions characterized by 1) inflammation, 2) vascular instability and 3) coagulation disturbances, which are all components of the endotheliopathy of trauma (EOT) (refs). Aside from their hemostatic properties, this proposal aims to also determine the mechanisms of action of the freeze dried platelets (FDPlts (Thrombosomes)) on the EOT in traumatic brain injury (TBI) and shock induced acute lung injury (ALI); all conditions with few if any effective treatment options.
We hypothesize that FDPlts (Thrombosomes) will have potent hemostatic properties comparable to fresh platelets and that they will attenuate and mitigate the endotheliopathy of trauma (EOT) in TBI and HS induced ALI. We hypothesize that FDPlts (Thrombosomes) can be used as a stand-alone early therapy to mitigate outcomes in trauma.

15. SUBJECT TERMS

TBI- Traumatic brain Injury
HS- Hemorrhagic Shock, ALI- Acute Lung Injury

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1. INTRODUCTION:

Currently in blood-banking practice in the US, platelets are stored in incubators at 22°C, with gentle agitation for up to 5 days. The main reason for this practice of storage at 22°C is to allow for adequate circulating numbers of platelets post transfusion and to avoid the risk of bacterial contamination. It has been shown that storage of platelets at 22°C for 5 days is associated with a decline in function of the platelets, also known as a storage lesion. One option is for blood banks to store platelets at 4°C, which is currently approved for 3 days of storage; however, diminished function of 4°C platelets has also been reported. Alternatively, a freeze-dried platelet (FDPlts) product can circumvent these challenges by providing hemostasis, prolonging the shelf life of platelets without cold storage and significantly enhancing the utilization and safety of transfused platelet units. FDPlts (Thrombosomes), made by Cellphire Inc., are an infusible freeze-dried platelet-derived hemostatic agent, stabilized with trehalose and polysucrose prior to and during freeze-drying. They can be stored at room temperature with prolonged shelf life (>1 yr), eliminating the need for bacterial testing, and logistically allow for platelet availability in remote and austere conditions.

Characterization studies demonstrate that FDPlts (Thrombosomes) express markers such as P-selectin and phosphatidylserine, hence indicating that they are activated. FDPlts (Thrombosomes) have demonstrated to have potent hemostatic properties. Canines undergoing coronary artery bypass grafting (CABG) treated with fresh platelets or FDPlts (Thrombosomes) showed a dose dependent decrease in blood loss. FDPlts (Thrombosomes) also deliver hemostatic efficacy in uncontrolled arterial bleeding in rats and New Zealand white rabbits (NZWR) with busulfan induced thrombocytopenia. Thus, FDPlts (Thrombosomes) are primed hemostatic agents that can be used towards the treatment of acute uncontrolled hemorrhage in bleeding patients. Safety studies with FDPlts (Thrombosomes) have been performed in several species including non- human primates and humans. No evidence of systemic thrombosis or non-specific thrombosis has been noted, which is a concern when utilizing an activated platelet product. **The goal of this project is to test the therapeutic potential of freeze dried platelets-FDPlts (Thrombosomes)- in disease conditions characterized by 1) inflammation, 2) vascular instability and 3) coagulation disturbances, which are all components of the endotheliopathy of trauma (EOT) (refs).** Aside from their hemostatic properties, this proposal aims to also determine the mechanisms of action of the freeze dried platelets (FDPlts (Thrombosomes)) on the EOT in traumatic brain injury (TBI) and shock induced acute lung injury (ALI); all conditions with few if any effective treatment options.

We hypothesize that FDPlts (Thrombosomes) will have potent hemostatic properties comparable to fresh platelets and that they will attenuate and mitigate the endotheliopathy of trauma (EOT) in TBI and HS induced ALI. We hypothesize that **FDPlts (Thrombosomes) can be used as a stand-alone early therapy** to mitigate outcomes in trauma.

2. KEYWORDS:

Hemorrhagic shock, Freeze-dried platelets, Thrombosomes, Traumatic brain injury, Inflammation, Vascular dysfunction, endotheliopathy of trauma

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Specific Aim 1: Characterize *in vitro* effects of Thrombosomes	**Months**	**UCSF (Pati)**
Major Task 1: Months 1-6: Obtain approval from institutional IACUC and ACURO for HS model.	100% complete	Dr. Pati
Milestone(s) Achieved: ACURO approval for HS and TBI model completed	6	
Major Task 2: FDP-Thrombosomes effects on endothelial permeability and signaling (Aim 1)		Dr. Pati
Subtask 1: Grow PECs (pulmonary endothelial cells) and brain endothelial cells (BECS) to sufficient quantities for *in vitro* assays Start ECIS and endothelial functional assays of platelet groups	3-6	100% completed
Subtask 2: Complete ECIS assays of platelet groups on all endothelial cells. Conduct Western Blots of endothelial signaling pathways and staining of ECs for junctional and cytoskeletal markers	7-9	*80% completed*
Subtask 3: Continue and complete Western Blots of endothelial signaling pathways and staining of endothelial cells for junctional markers.	10-12	
Milestone(s) Achieved: Comparison of Platelet groups on EC permeability and PEC and BEC signaling	12	
Major Task 3: Effect of FDP (Thrombosomes) on HS induced ALI- 3 hour model (Aim 2)-		Dr. Pati
Subtask 1: HS induced ALI Model acute three hour study (51 animals)	6-9	100% completed
Subtask 2: HS induced ALI Model acute three hour study (51 animals)	10-12	
Subtask 3: Sectioning of HS induced ALI 3 hour Model (102 animals)	13-15	100% completed
Subtask 4: Tissue analysis 3 hour HS model (102 animals)	16-18	100% completed
Milestone(s) Achieved: Determine efficacy and optimal dose of	18	

FDP in vivo to modulate EOT/ALI in 3 hour model of HS		
Major Task 4 Effect of FDP (Thrombosomes) on HS induced ALI-24 hour model (Aim 2)		Dr. Pati
Subtask 1: HS induced ALI Model 24 hour study (84 animals)	19-24	100% completed
Subtask 2: Sectioning of HS induced ALI 24 hour Model (84 animals)	25-27	
Subtask 3: Tissue analysis 24 hour HS model (84 animals)	28-30	
Milestone(s) Achieved: Determine efficacy and optimal dose of FDP in vivo to modulate EOT/ALI in 24 hour model of HS	27	
Major Task 5 Effect of FDP (Thrombosomes) in TBI (Aim 3)		Dr. Pati
Subtask 1 TBI – optimizing dose of FDP (perform surgeries and collect tissue at 3 day time point) – Total of 80 mice	13-16	100%
Subtask 2 - Tissue analysis (barrier permeability – 80 mice)	16-18	100%
Subtask 3 TBI – optimizing timing of delivery of FDP (perform surgeries and collect tissue at 1 day and 3 day time point) – Total of 70 mice	18-21	
Subtask 4 - Tissue analysis (sectioning and staining – 70 mice)	19-24	100%
Subtask 5 – Setting up behavior tests with control age and strain matched mice	18-24	10% completed
Subtask 6 –Behavior test optimization – data analysis	24-30	
Subtask 7 - TBI – Acute time point surgeries and tissue collection (168 mice)	25-31	100% completed
Subtask 8 TBI – Acute time point tissue sectioning, staining, and analysis (168 mice)	31-36	100% completed
Subtask 9 TBI – chronic time point surgeries and tissue collection, sectioning, staining (115 mice)	35-42	
Subtask 10 TBI – tissue analysis and behavior analysis for chronic time point mice (115 mice) and overall data analysis	40-48	
Milestone(s) Achieved: Complete studies of FDP effects in TBI.	48	
Major Task 6: Measure Thrombosome effects on endothelial glycocalyx and clot formation		Dr. Pati
Intravital Microscopy of mice (64 animals)	31-36	50% completed
Intravital Microscopy of mice (64 animals)	37-42	
Milestone(s) Achieved: Completion of testing for FDP effects on endothelial glycocalyx and clot formation	36	
Milestone(s) Achieved: Thrombosome production		
Major Task 7: Submit abstracts to meetings and manuscripts. Submit final report to DOD	48	Dr. Pati

What was accomplished under these goals?

The major work completed during this year was as follows:

1. *We have continued work with the 24 hour in vivo model of hemorrhagic shock. Here we found that the vascular dysfunction and inflammation related parameters which were observed at 3 hours post-shock, were resolved by 24 hours in vehicle treated mice. Please see the attached submitted and accepted manuscript for Shock.*
2. *We have carried out in vivo cremaster preparations to evaluate the effect of FDPlts on glycocalyx thickness.*
3. *We have developed a 3D organoid culture to look at effects of freeze-dried platelets and fresh apheresis platelets.*
4. *We have analyzed the effects of fresh apheresis platelets in traumatic brain injury model for blood brain barrier permeability.*
5. *We have continued studies on traumatic brain injury model and freeze-dried platelets (FDPlts) and fresh apheresis platelets (Plts). We have started the histological evaluation of tissue for markers of hemorrhage, neuroinflammation, infiltrating immune cells and gliosis.*

Methods:

Human platelets and freeze-dried platelets (FDPlts)

Units of leukoreduced apheresis platelets stored in plasma were obtained from Bonfils Blood Center , Denver, CO on Day 1. All platelets were tested for bacterial infection by the Bonfils Blood Bank and Blood Centers of the Pacific) and found to be negative. Freeze-dried platelets (FDPlts) were obtained from Cellphire, Inc.

BBB organoids formation

Primary human astrocytes were cultured in Astrocyte Growth Medium (ScienCell Research Laboratories) containing 2% FBS, astrocyte growth supplement and penicillin-streptomycin. Human brain microvascular pericytes (HBVP; ScienCell Research Laboratories) were cultured in Pericyte Medium containing 2% FBS, pericyte growth supplement and penicillin-streptomycin. Human cerebral microvascular ECs (hCMEC/D3) were maintained in culture using EndGRO^{TM} containing human endothelial growth factor, hydrocortisone, L-Glutamine, FBS, rhEGF, ascorbic acid and heparin. For BBB organoids formation, primary human astrocytes, HBVP and hCMEC/D3 were released by trypsin/EDTA and resuspended in hCMEC/D3 working medium. The concentration of each cell type was determined using a hemocytometer. $1.5x10^3$ of each cell type was seeded in each well of the 96-well plate coated with 1% agarose in a 1:1:1 ratio. Cells were placed in a humidified incubator at 37 °C with 5% CO_2 for 48–72 h to allow for the assembly of multicellular BBB spheroids.

Permeability assay

BBB organoids were incubated with TNFα (100ng/ml), and 10 μg/ml of FITC-Dextran (3kDa) in the medium for 5 h at 37 °C with 5% CO_2. Organoids were washed 3 times with PBS (5 min each),

fixed with 4% Paraformaldehyde, then transferred into a Frame-Deal™ incubation chambers (BIO-RAD) and imaged under a confocal microscope. Quantification of organoid permeability to fluorescent dextran was performed using ImageJ software. The mean fluorescence intensity of the core of each spheroid at 50-100 μm depth was quantified and plotted using GraphPad Prism.

Animal Protocols

The animal studies were performed with approval of the Institutional Animal Care and Use Committee at UCSF. The experiments were conducted in compliance with the National Institutes of Health guidelines on the use of laboratory animals and the Department of Defense Animal Care and Use Review Office. All animals were housed in a room with access to food and water ad libitum, controlled temperature and 12:12-hour light-dark cycles.

Cremaster Intra-vital Imaging

To induce a vascular injury, super perfusate with TNFa was dripped onto the cremaster prep and covered with thin plastic for ten minutes. Temperature was maintained by dripping warm super perfusate onto the plastic. At the same time of TNFa administration a bolus of 200 uL FFP, FDPlts, plts was made via IV. Shams were uninjured. After treatment a 100μL bolus of 2mg/ml FITC-tagged 70kD dextran in saline was administered via the cannula. Vessels were recorded for the next 30 minutes. Glycocalyx thickness was determined by comparing the width of the dextran column to the width of the vessels.

Traumatic Brain Injury (TBI) model

The controlled cortical impact model (CCI) of traumatic brain injury (TBI) was performed on 10-12 week old C57Bl/6J mice (Jackson Laboratories, Sacramento, CA). Briefly, isofluorane-anesthetized mice received a 5mm craniotomy and an impact was made using Leica Impactor 1 on the right parietal cortex, with the following setting to generate a moderate-severe injury level. A 3mm piston impacted the cortex at a velocity of 4.5 mm/s, to a depth of 1.2 mm, with a dwell time of 300 ms. Animals were injected with saline or FDPlts ($1.5x10^6$ particles/g body weight) at two hours and twenty four hours post-injury. Blood brain barrier permeability was evaluated at 3 days post-injury. An hour before sacrifice, animals were retro-orbitally injected with IR dye conjugated-10 kD dextran and allowed to circulate. At the end of the hour circulation, mice were transcardially perfused with ice cold PBS and brains were then removed and sliced at 2mm thickness. Dye intensity was quantitated using the Odyssey imaging system (Licor, NY). Brain slices were then fixed with 4% PFA overnight and dehydrated for 3-5 days in 30% sucrose solution then embedded in OCT. Embedded brains were further sectioned to 20 μm thickness using a Leica 1950 cryostat (Leica Biosystems, Wetzlar, Germany).

Processing

Brain slices that were scanned on Licor for blood brain barrier (BBB) permeability were fixed with 4% paraformaldehyde (PFA) overnight and dehydrated for 3-5 days in 30% sucrose solution then embedded in OCT. Embedded brains were further sectioned coronally to 20 μm thickness using a Leica 1950 cryostat (Leica Biosystems, Wetzlar, Germany) and sections collected on Superfrost slides (ThermoFisher, Waltham, MA).

Immunostaining, Imaging, and Quantitation

PFA fixed Brain sections were stained with antibodies against GR1 (neutrophil marker, BD Biosciences, San Jose, CA), CD68 (macrophage marker, Bio-Rad, Hercules, CA), GFAP (Astrocyte marker, Millipore, Burlington, MA), Iba-1 (Microglia marker, Novus Biologicals, Littleton, CO) and Ter119 (RBC marker for hemorrhage, BD Pharmingen). Fluorescently tagged secondary antibodies were used for detection. Images were captured using a Nikon Eclipse 80i microscope (Nikon, Melville, NY) with RT-scmos camera (SPOT Imaging, Sterling Heights, MI) at 10x magnification using same exposure settings across all animals for each of the individual markers. Two slides per animal, with three sections per slide and 2-7 images per section were analyzed. For GR1 analysis, each section was given a score as follows: score 0 = 0-5 neutrophils in the whole sections, score 1 = less than 10 in whole section, score 2 = 5-10/field. These scores were averaged per section and then totaled per animal. For all the other markers, fluorescent intensity measurements were performed. Integrated fluorescence intensity was measured using ImageJ software and Auto Threshold (MaxEntropy method). Percentage integrated fluorescence density was calculated as (Threshold fluorescence intensity/Total fluorescence intensity) x 100. Statistical comparison were analyzed using two-tailed student's t-test for normally distributed data and non-parametric test for data that were not normally distributed using Graphpad Prism software.

Statistical analyses and power calculation

Statistical analyses were performed using Prism 8.3 software (Graphpad Inc. San Diego, CA), multiple group comparisons were determined by One way ANOVA with post-hoc Tukey tests for all in vitro experiments. In vivo experiments were analyzed by RM two way ANOVA. An α of 0.05 was preset as the cutoff for statistical significance. All data are represented as mean ± SD.

Results:

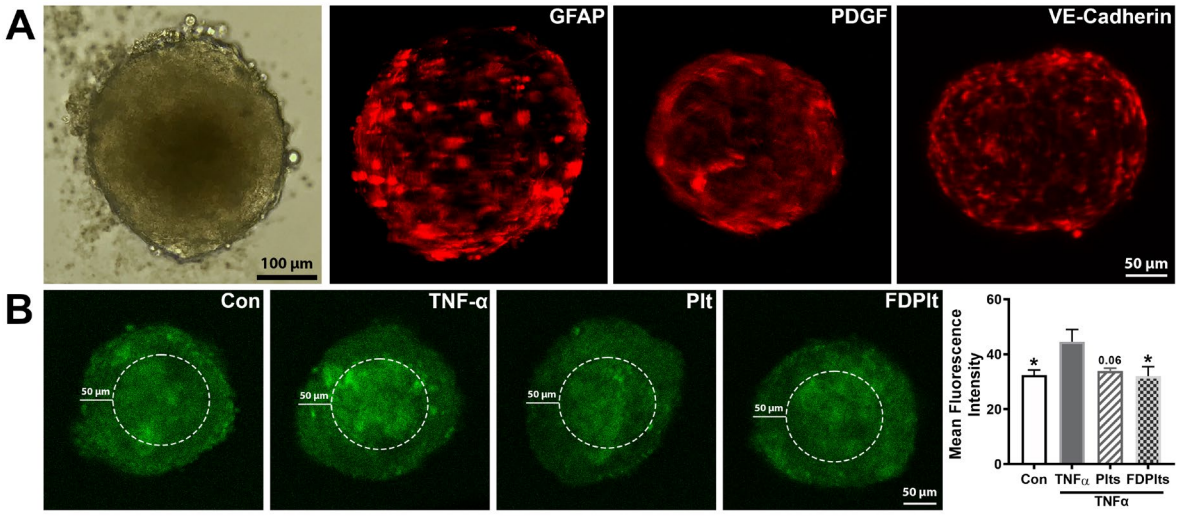


Figure 1: FDPlts restore TNFα induced dextran permeability in 3D organoids to levels of control, but Plts do not. A) Panel showing representative images of 3D organoid in bright field consisting of brain vascular endothelial cells (VE-cadherin positive), pericytes (PDGF positive) and astrocytes (GFAP positive) **B)** Organoids were treated with TNFα (100ng/ml), and 10 µg/ml of FITC-Dextran (3kDa) in the medium for 5 h at 37 °C with 5% CO_2. One way ANOVA, p=0.0005; Holm Sidaks multiple comparison test as indicated on the graph. *p<0.05

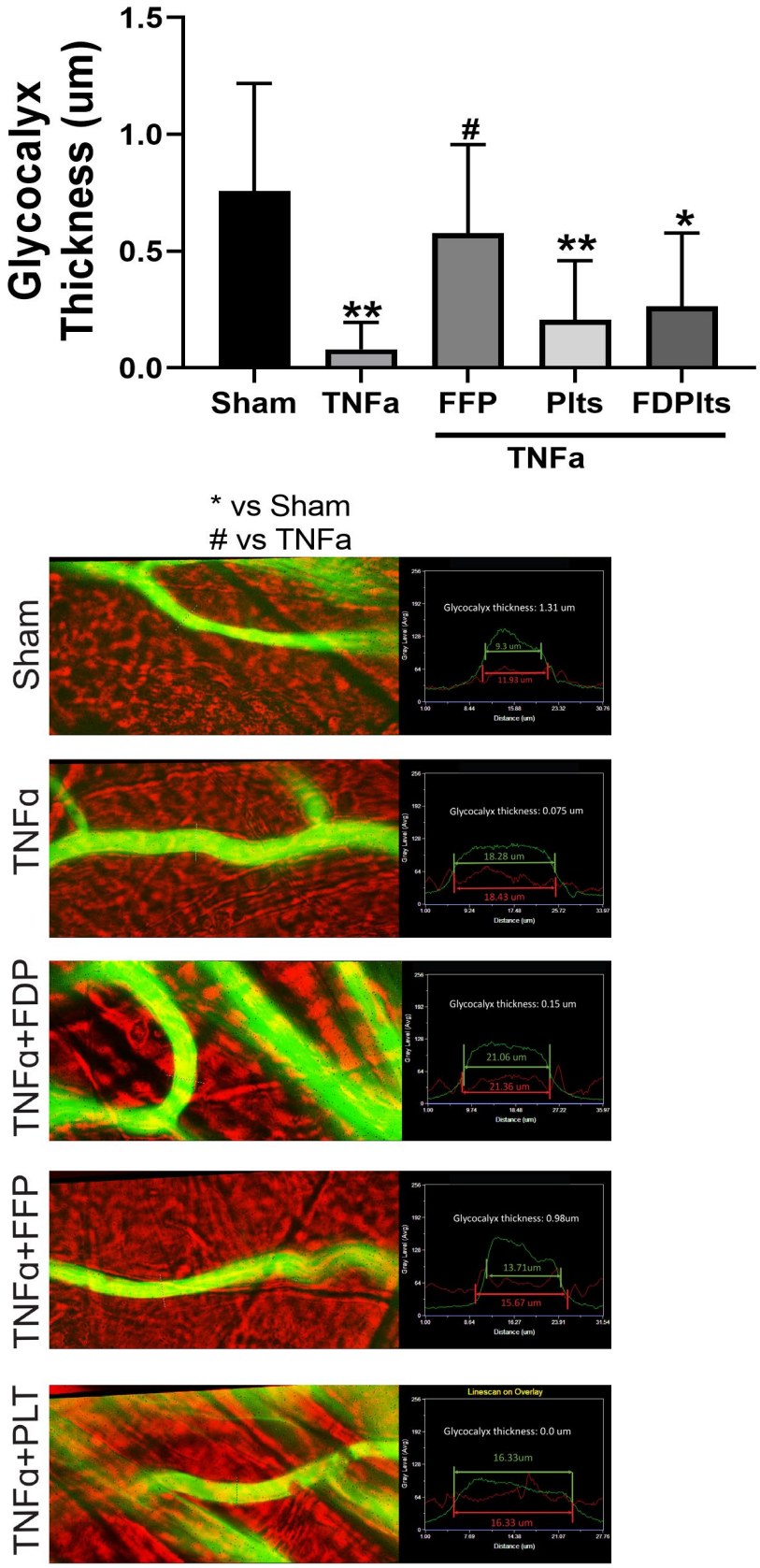


Figure 2: FFP restores TNFa induced glycocalyx loss to levels of sham, but Plts or FDPlts do not. Panel showing representative images of blood vessels and outline of glycocalyx thickness measurement with each of the treatments indicated. Graph represents analysis of glycocalyx thickness. One way ANOVA, p=0.0005; Holm Sidaks multiple comparison test as indicated on the graph. *p<0.05, **p<0.01.

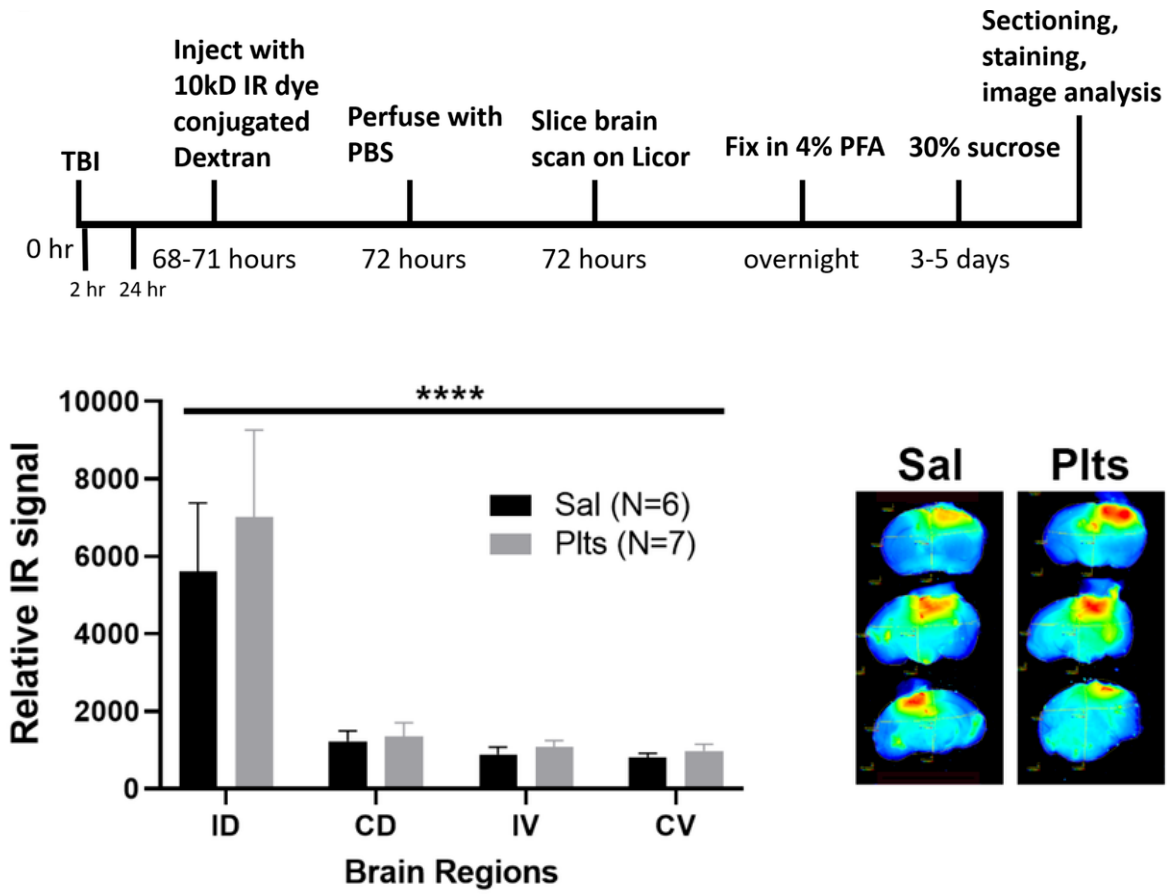


Figure 3: Treatment with Plts does not affect Barrier Disruption at 3 days post-injury
Mice were subjected to TBI and treated with either saline or Plts at 2h and 24 h post-injury and barrier permeability was measured at 3 days post-injury using a 10kD IR-680 tagged dextran. Both the saline and Plts treated groups show focal increased barrier permeability in the ipsilateral dorsal quadrant as compared to the other three quadrants and the Plts treatment did not affect permeability. ID- Ipsilateral Dorsal, IV – Ipsilateral Ventral, CD- Contralateral Dorsal, CV- Contralateral Ventral. Repeated measures two-way ANOVA showing a significant effect of region (ID>all other regions) $p<0.0001$.

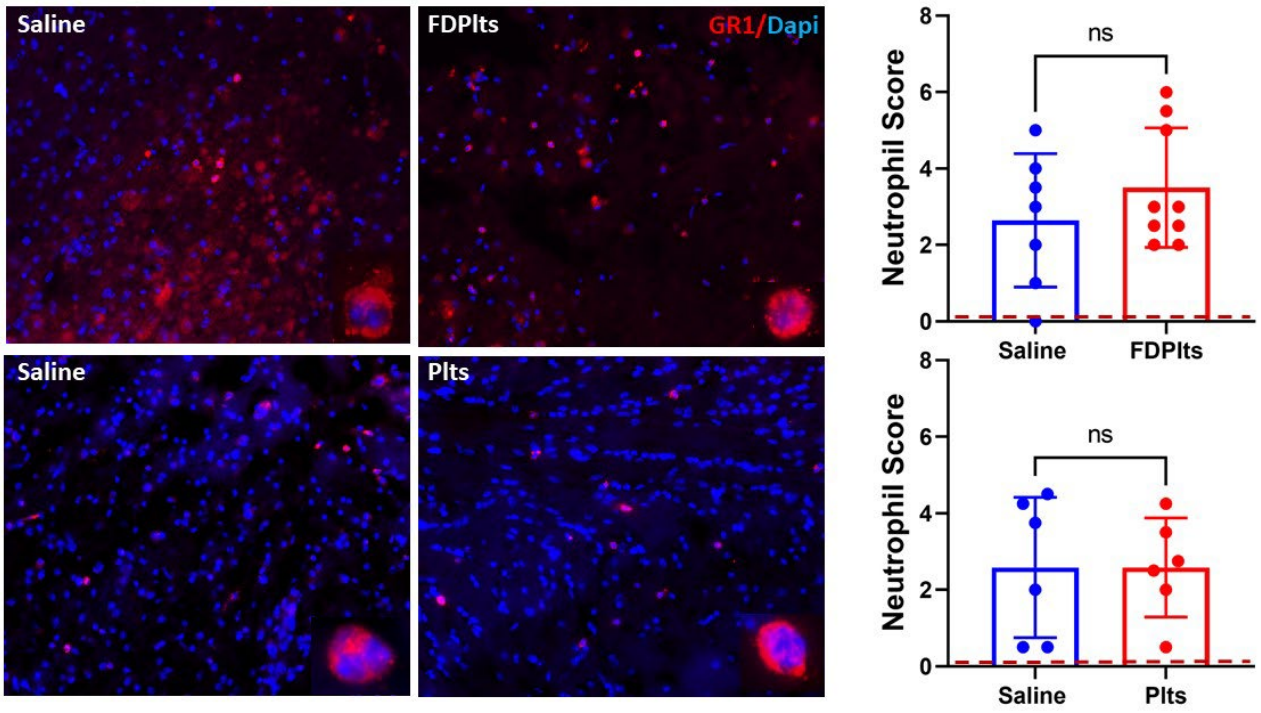


Figure 4: Treatment with FDPlts or Plts does not affect neutrophil infiltration at 3 days post-injury
Mice were subjected to TBI and treated with either saline, FDPlts or Plts at 2h and 24 h post-injury. Brain slices were sectioned and stained with GR1 (marker of neutrophils). Sections were scored for infiltration as described in methods section. There were no differences between FDPlts or Plts treated groups as compared to their respective saline treated animals. Images represent animals that have the average score for each of the treatment groups, in the images red cells depict, GR-1 positive cells and blue nuclei (Dapi positive), inset shows high magnification images that show lobulated nuclei. Bar graphs represent mean ± SEM, with individual animals as symbols, dashed red line represents value of sham animals. Two-tailed t-test ($p>0.05$).

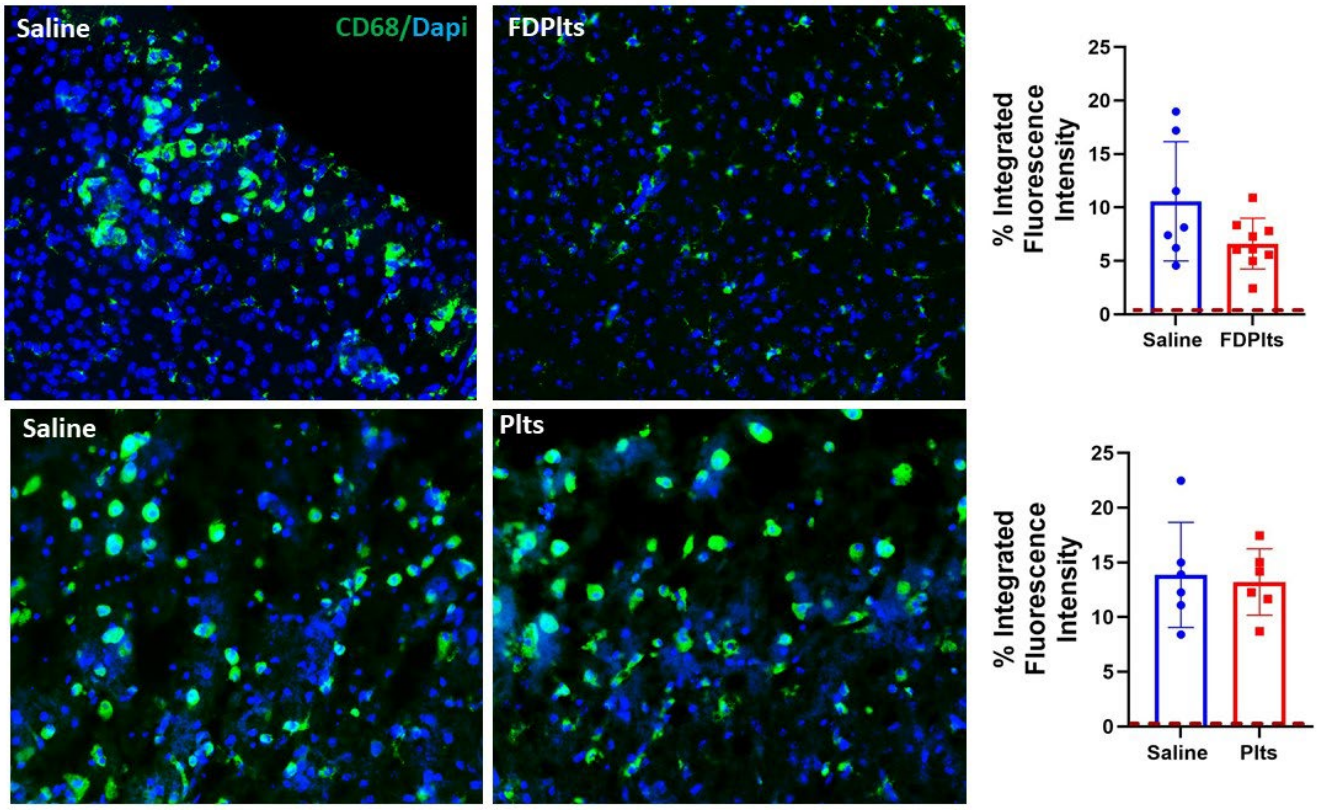


Figure 5: Treatment with FDPlts or Plts does not affect macrophage infiltration at 3 days post-injury
Mice were subjected to TBI and treated with either saline, FDPlts or Plts at 2h and 24 h post-injury. Brain slices were sectioned and stained with CD68 (marker of macrophages). Sections were analyzed using ImageJ for infiltration as described in methods section. There were no differences between FDPlts or Plts treated groups as compared to their respective saline treated animals. Images represent animals that have the average score for each of the treatment groups, in the images green cells depict, CD68 positive cells and blue nuclei (Dapi positive). Bar graphs represent mean ± SEM, with individual animals as symbols, dashed red line represents value of sham animals. Two-tailed t-test ($p>0.05$).

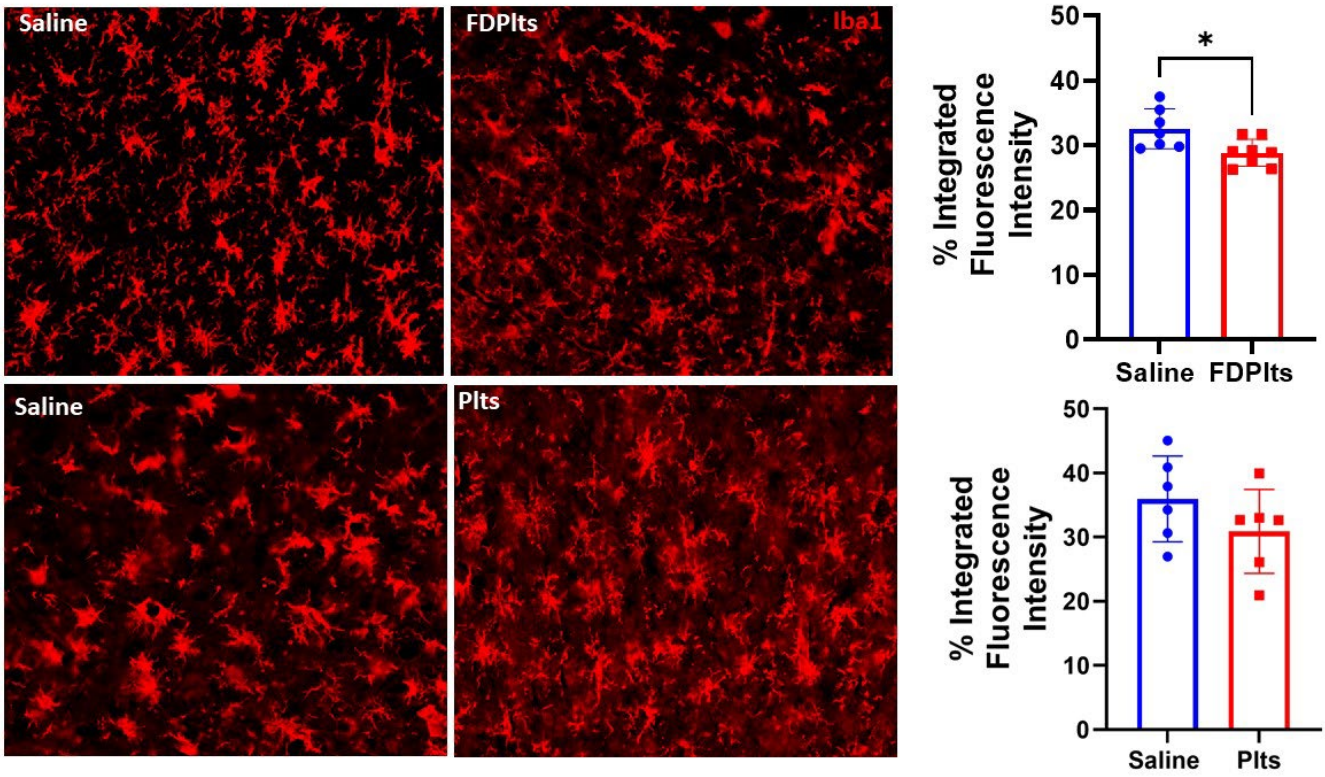


Figure 6: Treatment with FDPlts reduces microglial response but Plts treatment does not affect microglial response as compared to their respective saline treated controls at 3 days post-injury

Mice were subjected to TBI and treated with either saline, FDPlts or Plts at 2h and 24 h post-injury. Brain slices were sectioned and stained with Iba1 (marker of microglia). Sections were analyzed using ImageJ for activation as described in methods section. FDPlts treatment significantly reduced injury induced (saline treated) microglial response in the cortex, but Plts treated group did not change as compared to their respective saline treated animals. Images represent animals that have the average score for each of the treatment groups, in the images red cells depict, Iba1 positive cells. Bar graphs represent mean ± SEM, with individual animals as symbols. Two-tailed t-test (FDPlts vs saline, *$p<0.05$; Plts vs saline, $p>0.05$).

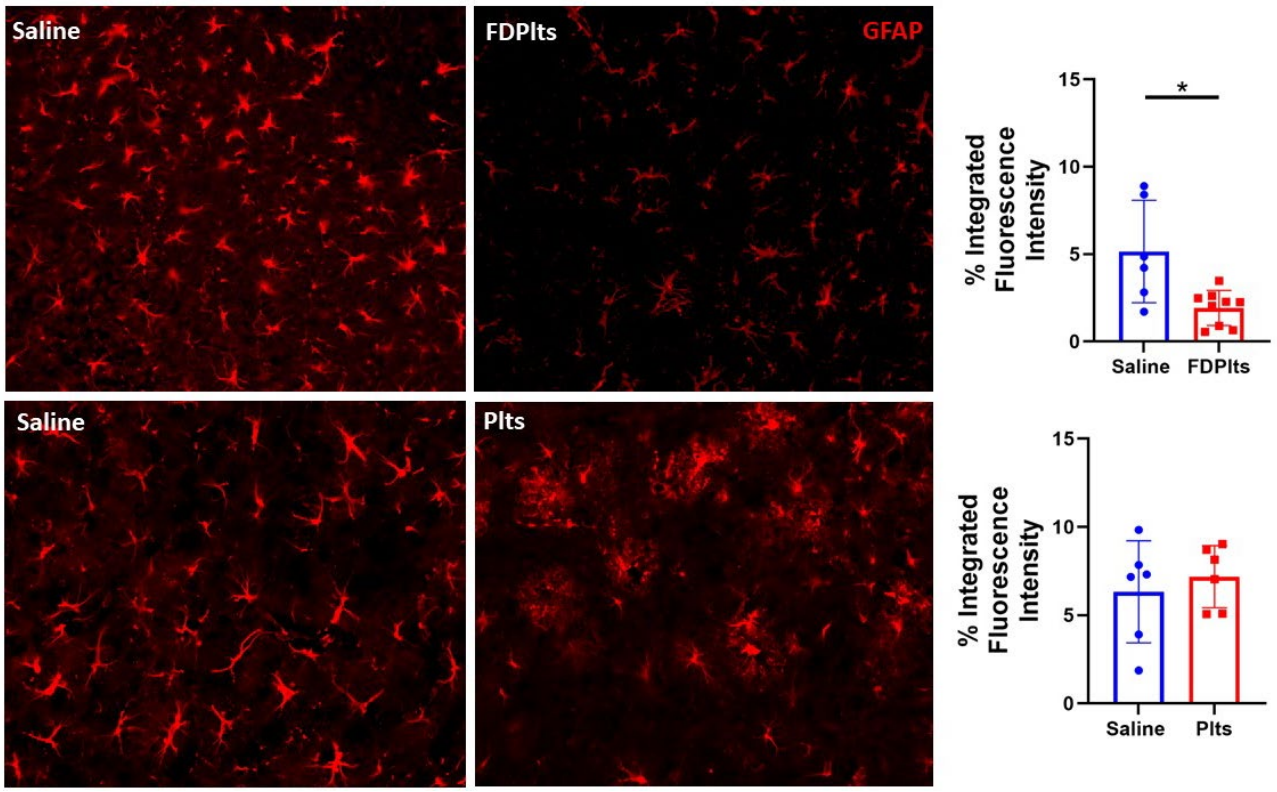


Figure 7: Treatment with FDPlts reduces astroglial response but Plts treatment does not affect astroglial response as compared to their respective saline treated controls at 3 days post-injury
Mice were subjected to TBI and treated with either saline, FDPlts or Plts at 2h and 24 h post-injury. Brain slices were sectioned and stained with GFAP (marker of astroglia). Sections were analyzed using ImageJ for activation as described in methods section. FDPlts treatment significantly reduced injury induced (saline treated) astroglial response in the cortex, but Plts treated group did not change as compared to their respective saline treated animals. Images represent animals that have the average score for each of the treatment groups, in the images red cells depict, GFAP positive cells. Bar graphs represent mean ± SEM, with individual animals as symbols. Two-tailed t-test (FDPlts vs saline, *$p<0.05$; Plts vs saline, $p>0.05$).

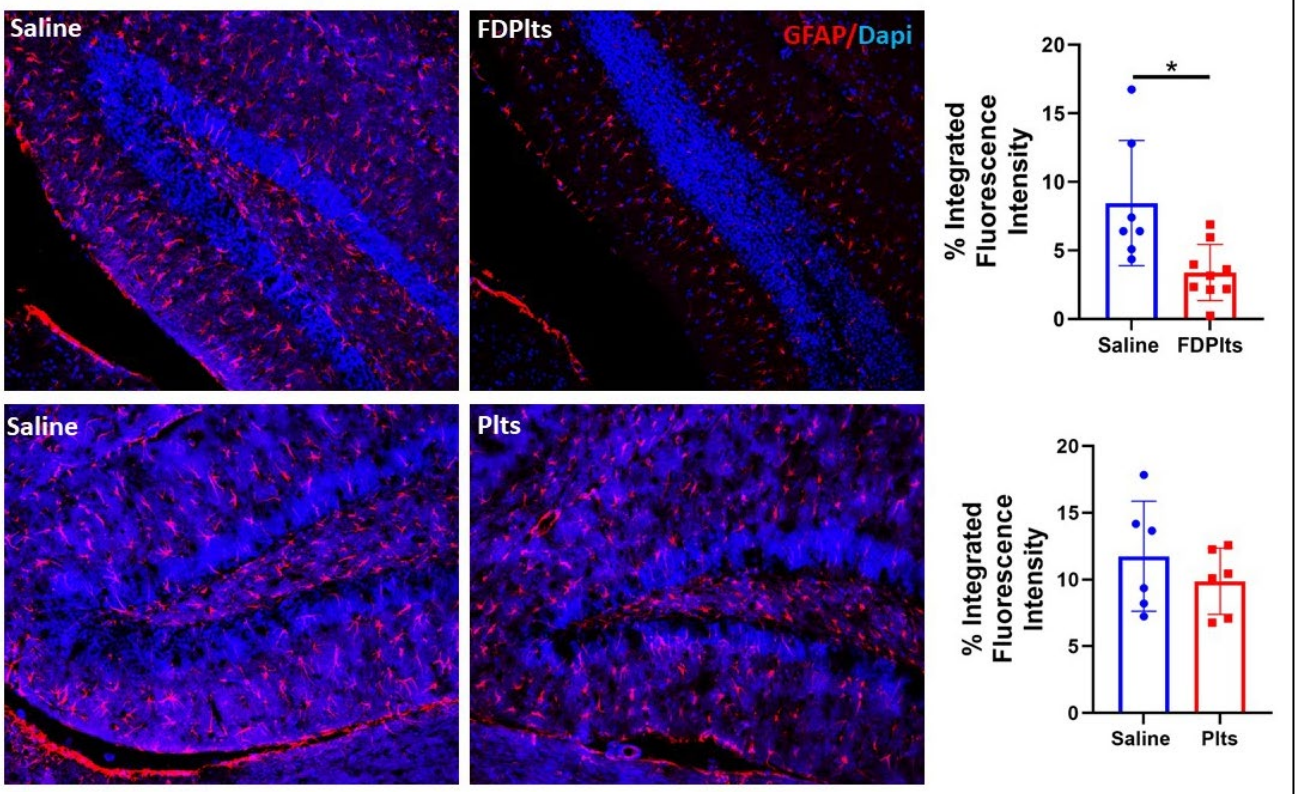


Figure 8: Treatment with FDPlts reduces astroglial response but Plts treatment does not affect astroglial response as compared to their respective saline treated controls at 3 days post-injury
Mice were subjected to TBI and treated with either saline, FDPlts or Plts at 2h and 24 h post-injury. Brain slices were sectioned and stained with GFAP (marker of astroglia). Sections were analyzed using ImageJ for activation as described in methods section. FDPlts treatment significantly reduced injury induced (saline treated) astroglial response in the hippocampus, but Plts treated group did not change as compared to their respective saline treated animals. Images represent animals that have the average score for each of the treatment groups, in the images red cells depict, GFAP positive cells. Bar graphs represent mean ± SEM, with individual animals as symbols. Two-tailed t-test (FDPlts vs saline, *$p<0.05$; Plts vs saline, $p>0.05$).

What opportunities for training and professional development has the project provided?

Lindsay Vivona, a research associate was able to present this work at an international meeting of Shock.

How were the results disseminated to communities of interest?

This work in a 24h mouse model of hemorrhagic shock was presented at the Shock conference (June 2022) and is accepted to be published in the journal Shock (see Appendix).

What do you plan to do during the next reporting period to accomplish the goals?

- We will continue the cell culture experiments to examine the signaling cascade of endothelial cell and platelet and freeze dried platelet cross talk (Major task 2, subtasks 2 and 3).
- Start behavior study set up run of mice (Major task 5, subtask 6)
- Continue studies in traumatic brain injury – evaluation of the effect of freeze dried platelets on long-term behavioral outcomes (Major task 5)

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

In our 24h hemorrhagic shock animal model, we found that the inflammatory and vascular response resolve at 24h post-injury. In the traumatic brain injury model, we found that only the freeze dried platelets protect the blood brain barrier. Similar finding were reproduced in an in vitro endothelial cell culture as well as 3D organoid model. Fresh apheresis platelets did not show a similar effect.

What was the impact on other disciplines?

This finding could be of great utility for storage and availability of platelets in remote and austere environments and also be a safer alternative from a infectious stand point of bacterial contamination. Eventually this could change Blood Banking practice in the US and military settings.

What was the impact on technology transfer?

Nothing to report.

What was the impact on society beyond science and technology?

Nothing to Report

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change

Nothing to Report

Actual or anticipated problems or delays and actions or plans to resolve them

1. Due to space constraints, the behavior room was reestablished at UCSF. We have set up behavior room in the barrier facility with new equipment and will now set up all tests.

Changes that had a significant impact on expenditures

Nothing to Report

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Significant changes in use or care of human subjects

Not applicable

Significant changes in use or care of vertebrate animals

None

Significant changes in use of biohazards and/or select agents

None

6. PRODUCTS:

- **Publications, conference papers, and presentations**
  Journal publications.

Our second manuscript is accepted for publication in Shock (Accepted manuscript is attached in the appendix).

Books or other non-periodical, one-time publications.

Nothing to Report

Other publications, conference papers and presentations.

*This is a paper that is focused on the development of the 24 hour model in which we planned to test the Thrombosomes on chronic injury from hemorrhagic shock and trauma. Interestingly the model does not demonstrate marked changes in the lungs or small intestine as it does in the 3 hour model. Since this is important to the field for this established model, we submitted our data. It is in press now in Shock.

RECOVERY OF ENDOTHELIOPATY AT 24 HOURS IN AN ESTABLISHED MOUSE MODEL OF HEMORRHAGIC SHOCK AND TRAUMA

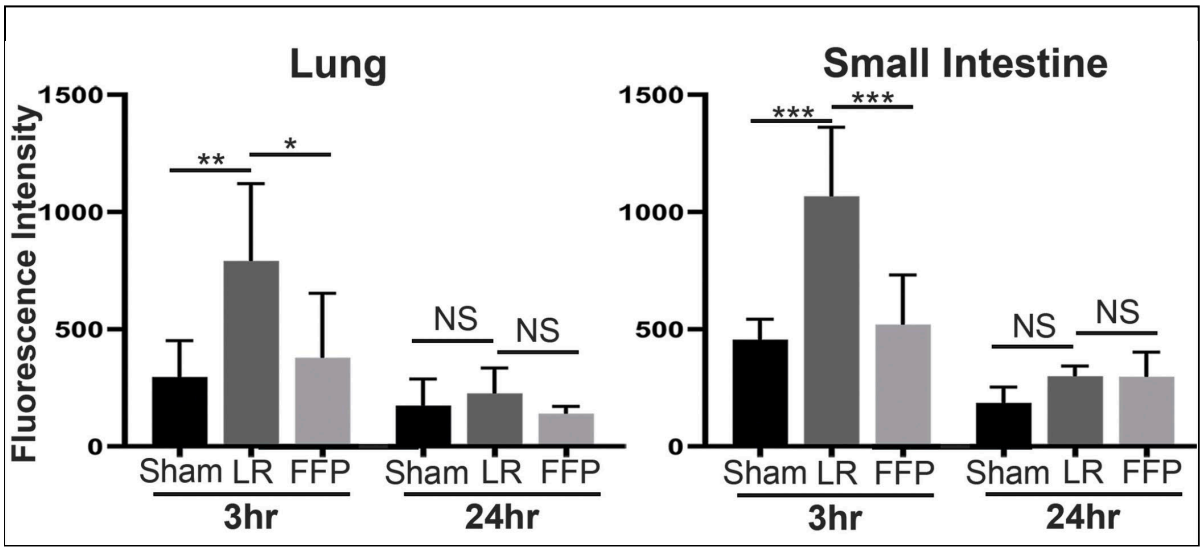
Mark Barry MD[1], Alpa Trivedi PhD[2], Lindsay Vivona BS[2], Jenna Chui[2], Byron Miyazawa BS[2], Praneeti Pathipati PhD[2], Shibani Pati MD, PhD[2]*

Introduction: The endotheliopathy of trauma develops early after injury and consists of increased vascular permeability, inflammation, and dysfunctional coagulation. Persistence of these abnormalities ultimately leads to multi-organ failure. We hypothesized that extending an established acute mouse model of hemorrhagic shock and trauma (HS/T) to a 24-hour survival model would allow for evaluation of persistent endotheliopathy and organ injury after HS/T.

Methods: Adult male C57BL/6J mice underwent laparotomy, femoral artery cannulation, and blood withdrawal to induce HS to a mean arterial pressure of 35mmHg for 90 minutes. Mice were resuscitated with either lactated Ringer's (LR) or fresh frozen plasma (FFP). Vascular permeability in the lung and gut were assessed by measuring extravasation of a fluorescent dextran dye. Lungs were evaluated for histopathologic injury, and immunofluorescent staining was used to evaluate neutrophil infiltration and intercellular junction integrity. Pulmonary inflammatory gene expression was evaluated using NanoString. All endpoints were evaluated at both 3 and 24 hours after initiation of shock.

Results: LR- and FFP-treated mice had an equal mortality rate of 17% in the 24-hour model. LR-treated mice demonstrated increased vascular permeability in the lung and gut at 3 hours compared to sham mice (lung $P < .01$; gut $P < .001$), which was mitigated by FFP treatment (lung $P < .05$; gut $P < .001$). Twenty-four hours after shock, however, there were no differences in vascular permeability between groups. Similarly, the lungs of LR-treated mice demonstrated significant histopathologic injury, neutrophil infiltration, loss of tight and adherens junctions, and a pro-inflammatory gene expression profile at 3 hours, all of which recovered by 24 hours.

Conclusions: In an established mouse model of HS/T, endotheliopathy and lung injury are evident at 3 hours but recover by 24 hours. Larger animal models allowing for more severe injury coupled with supportive care are likely necessary to evaluate endotheliopathy and organ injury outside of the acute period.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- **Website(s) or other Internet site(s)**

Nothing to Report

- **Technologies or techniques**

Nothing to Report

- **Inventions, patent applications, and/or licenses**

Nothing to Report

- **Other Products**

Nothing to Report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Pati, Shibani	22.97%
Mahuvakar, Alpa	29.16%
Miyazawa, Byron	34.18%
Zhang, Haoqian	11.67%

Name: Shibani Pati MD PhD
Project Role: Principal Investigator
Nearest person month worked: 2.74 cal months
Contribution to Project: Dr.Pati is the principal investigator on this grant and has been overseeing the planning, data analysis and execution of the entire grant.

Name: Alpa Mahuvakar PhD.
Project Role: Research Scientist
Nearest person month worked: 2.43 cal months
Contribution to Project: Dr. Mahuvakar is a co-investigator and is involved in planning of studies, analysis of data, running/execution of all research experiments, including in vitro endothelial cell signaling and traumatic brain injury.

Name: Byron Miyazawa
Project Role: Research Associate
Nearest person month worked: 4.1 cal months
Contribution to Project: Running/execution of platelet assays, endothelial assays, staining of endothelium and signaling analysis

Name: Haoqian Zhang PhD.
Project Role: Research Scientist
Nearest person month worked: 3.92 cal months
Contribution to Project: In vitro blood brain barrier organoid assays and intravital imaging

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

None

What other organizations were involved as partners?

Cellphire Inc. Rockville MD
Cellphire provided the Thrombosome product needed for these studies.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: QUAD CHARTS:

9. APPENDICES: