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**TITLE:** **Targeting the Endotheliopathy of Trauma in Hemorrhagic Shock and Traumatic Brain Injury with Freeze-Dried Platelets**

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**CONTRACTING ORGANIZATION:** **University of California, San Francisco, CA**

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**14. ABSTRACT**

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 | *20% 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 | 50% 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 | 50% completed |
| Subtask 6 –Behavior test optimization – data analysis | 24-30 | |
| Subtask 7 - TBI – Acute time point surgeries and tissue collection (168 mice) | 25-31 | |
| Subtask 8 TBI – Acute time point tissue sectioning, staining, and analysis (168 mice) | 31-36 | |
| 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 | 20% 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 time was as follows:

1. Even under limited working capacity due to COVID19 restrictions, we have completed the acute time point studies with FDPlts in mouse model of hemorrhagic shock. This work was presented at the AAST conference (September 2020) and published in Journal of Trauma and Acute Care (see Appendix).
2. We have started in vitro endothelial signaling pathway analysis using FDPlts (Thrombosomes) and fresh platelets as controls.
3. We have completed traumatic brain injury (TBI) experiments with FDPlts (Thrombosomes) and fresh platelets as controls for barrier permeability analysis.
4. Due to space constraints, the behavior room was reestablished at UCSF. We have all the equipment now set up in the behavior room in the barrier facility.

**Methods:**

**Cell Culture**

Human umbilical cord derived endothelial cells (HUVECs) and brain derived endothelial cells (BECs) were grown under standard tissue culture conditions in 5% CO2.

**Human platelets and freeze-dried platetets (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.

**Transendothelial electric resistance (TEER)** The integrity of HUVEC and BEC monolayers was measured using an electric cell-substrate impedance sensing system (ECIS 1600, Applied BioPhysics, Troy, NY). An increase or decline in TEER across the cell monolayers indicated accordingly, decreased or increased endothelial paracellular permeability. Cells were grown to confluence on L-cysteine reduced, Fibronectin-pre-coated 96-well plates containing electrodes in each well. Cells were treated with platelets or FDPlts ($50x10^3$/µl- in 200 µL EBM-2 medium) and then challenged with thrombin. Monolayer resistance at 4/16/64 kHz was analyzed in 5-min intervals. Data were normalized to the mean resistance of cell monolayers before the treatments and plotted as area under the curve. 4- wells were tested/group. Statistical analysis conducted was by by standard Student –t test and one way ANOVA for comparison between groups.

**Immunostaining of endothelial tight and adherens junction proteins**

HUVECs were grown to confluence on collagen coated coverslips prior to treatment. FDPlts were reconstituted in pyrogen free, sterile water and added to the HUVEC monolayer at a concentration of $50x10^6$ particles/ml for 30 minutes, followed by a 0.2U/ml thrombin challenge for 5 minutes at 37°C. Plts were added in the same concentrations. Junction proteins VE-cadherin (Cell Signaling, Danvers, MA), and zonula occludens-1 (ZO-1) (Invitrogen, Carlsbad, CA) were stained. F-actin was detected with Texas Red Phalloidin (Cell Signaling). Three coverslips per treatment group were prepared, and four images were captured per coverslip.

Images were captured at 20x magnification using the Nikon Eclipse 80i microscope (Nikon, Melville, NY) with SPOT RT-sCMOS camera (SPOT Imaging, Sterling Heights, MI). Gap junction measurements were performed using ImageJ, an open source platform for biological image analysis.

**2-Dimensional Difference in Gel Electrophoresis (2D-DIGE) and Mass Spectrometry**

Thrombosomes and fresh platelets (Day 1 plts) were sent to Applied Biomics (Hayward, California) for 2-Dimensional Difference in Gel Electrophoresis (2D-DIGE) and mass spectrometry analysis. Samples were matched as pairs, and labeled with CyDye2 or CyDye5, to enable simultaneous co-separation and analysis of samples on a single multiplexed gel. 2D gel electrophoresis was used to separate the labeled samples on a single 2D gel using isoelectric focusing and SDS polyacrylamide gel electrophoresis (SDS-PAGE). ImageQuant and DeCyder computer software was used to quantify the difference in protein ratios between samples in order to identify protein differences between the matched pair. The EttanTM spot picker (GE Healthcare Life Systems, >99.9% accuracy) was then used to identify spots that represent proteins for which there existed significant differences. A p-value of $<0.05$ was used to identify proteins of interest. Selected spots were then identified using spectrometry matrix-assisted laser desorption/ionization time-of-flight/time-of-flight (MALDI-TOF/TOF). Extracted peptides were spotted into wells of a MALDI plate, and mass spectrometry was performed using Applied Biomics Proteomics Analyzer. The 10-20 most abundant peptides were then further fragmented and tandem mass spectrometry was performed to identify the proteins. Specific protein identification was performed by matching peptide mass mapping and peptide fragmentation mapping in primary sequence databases.

**Scanning Electron Microscopy**

FDPlts were reconstituted then washed and fixed in 3% gluteraldehyde, followed by serial dehydration in 50%, 70%, 90%, and 100% ethanol, and mounted onto ITO CTD coverslips (SPI, NY), then coated with gold-palladium (3nm). FDPlts were examined and imaged using an SEM (S4300, Hitachi, Tokyo, Japan) with an electron energy of 1.0–1.5 keV, at 1000x and 10,000x magnification.

**Western Blot Analysis**

Western blot experiments were carried out as previously described (Gibb, et al, 2015). Briefly, an equal number of platelets and FDPlts were collected, and lysed with RIPA lysis buffer. Resulting lysates were denatured with NuPage LDS sample buffer (Invitrogen, NY), and separated by SDS-PAGE, transferred to polyvinylidene difluoride membranes, and blotted with anti-tubulin (T6199) and anti-acetylated tubulin (T7451), (Sigma, St. Louis, MO) and IR dye-conjugated secondary antibodies (Licor, NY). Protein was detected and intensity quantitated using the Odyssey imaging system (Licor, NY).

**Flow Cytometry**

FDPlts were characterized by size and expression of human integrin-a2b (CD41) (Biolegend, San Diego, CA) and glycoprotein1b (CD42b) (Biolegend, San Diego, CA). Surface levels of P-selectin (CD62P) (Biolegend, San Diego, CA) were compared using the geometric mean fluorescence intensity (gMFI) of expression. All events were analyzed using computer software (FlowJo, Version 9.7, Tree Star, Inc., Ashland, OR).

**Protein Analysis - Luminex Assay**

A Luminex panel targeting the proteins angiogenin, angiopoietin-1, BDNF, CD40 ligand, FGF, IFN-γ, IL-1β, IL-6, IL-8, IL-10, PDGF-AA, PDGF-BB, thrombomodulin, thrombospondin-2, Tie-2, TNF-α, VEGF, and vWF-A2 was created (R&D Systems, Minneapolis, MN). FDPlts and day one platelets reconstituted and pelleted and lysed with RIPA buffer containing HALT, and diluted using diluents supplied by the manufacturer. This multiple analyte assay was performed using a MAGPIX instrument, and the resulting data were analyzed using xPONENT software.

**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.

**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).

**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 aANOVA. An $\alpha$ of 0.05 was preset as the cutoff for statistical significance. All data are represented as mean ± SD.

**Results:**

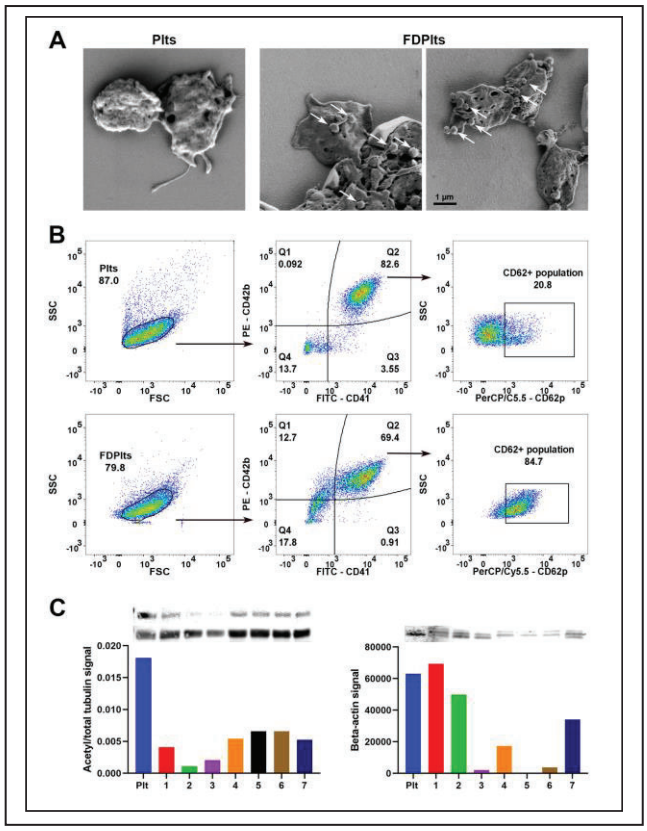



**Figure 1: In vitro characterization of FDPlts:** A) SEM imaging shows that FDPlts have activated morphology and presence of exposed extracellular vesicles as compared to day 1 platelets. B) Flow cytometry analysis demonstrates an increased expression of CD62p on FDPlts as compared to day 1 fresh platelets. C) Deacetylation of tubulin is a marker of platelet activation. Here we see increased deacetylation on freeze dried platelets (across multiple lots) as compared to day 1 platelets.

| | Plt | FDPlt | | | |
|---|---|---|---|---|---|
| | | CT1 | CT2 | CT3 | NT |
| Ang1 | 88038.4 | 98771.6 | 104446.2 | 103060.5 | 142533.9 |
| Ang2 | N/A | 667.0 | 724.5 | 731.7 | 968.5 |
| BDNF | 37233.7 | 94509.9 | 98948.5 | 96882.0 | 104522.4 |
| vWF-A2 | 418.9 | 567.5 | 682.4 | 657.8 | 927.6 |
| Thrombo Modulin | 3647.5 | 6679.0 | 7407.0 | 7004.8 | 8306.7 |
| Thrombo spondin2 | 1179.4 | 4065.0 | 4421.1 | 4131.8 | 4020.5 |
| CD40 | 14745.6 | 16458.2 | 17447.9 | 16880.1 | 19359.9 |
| PDGF-AA | 6185.1 | 14500.5 | 16488.8 | 15859.1 | 15265.3 |
| PDGF-BB | 30314.3 | 83742.4 | 99735.0 | 99660.0 | 99831.4 |
| VEGF | 315.7 | 352.2 | 399.1 | 370.3 | 627.9 |
| EGF | 36.0 | 236.4 | 328.5 | 251.6 | 351.3 |
| FGF | 250.5 | 75.7 | 95.3 | 93.3 | 102.6 |
| TNFα | 23.1 | 24.4 | 28.5 | 25.8 | 29.9 |
| IFNγ | 619.4 | 329.7 | 395.0 | 385.7 | 610.1 |
| IL-1β | 53.0 | 38.9 | 40.7 | 42.5 | 55.7 |
| IL-6 | 34.5 | 23.5 | 25.3 | 26.2 | 36.4 |
| IL-10 | 5.5 | 7.8 | 12.3 | 10.9 | 12.0 |
| CXCL8 | 33.8 | 52.3 | 65.1 | 62.1 | 63.6 |
| Angiogenin | 7778.2 | 18194.0 | 19958.8 | 19014.2 | 19429.2 |
| Tie2 | 660.7 | 832.5 | 919.8 | 882.2 | 907.2 |

**Table 1:** Factor content in the Thrombosomes is preserved irrespective of the freeze drying process and change in morphology. More importantly, they are enriched in potent vascular regulators and growth factors, as seen in the graphical representation below.

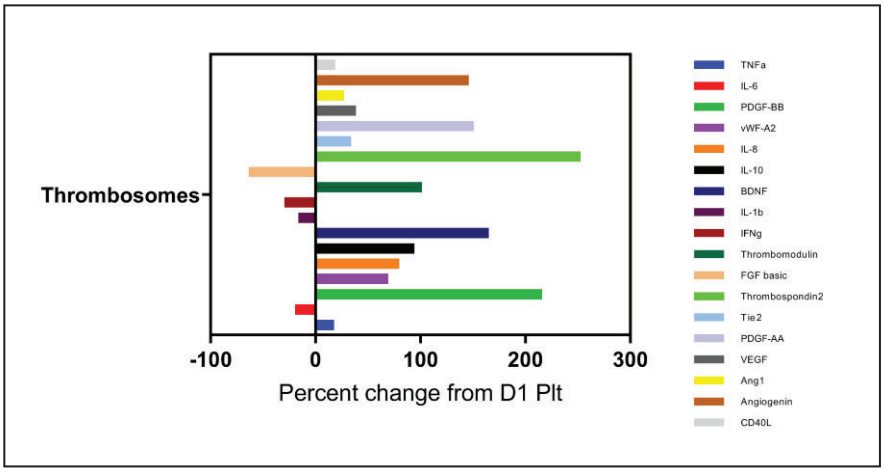
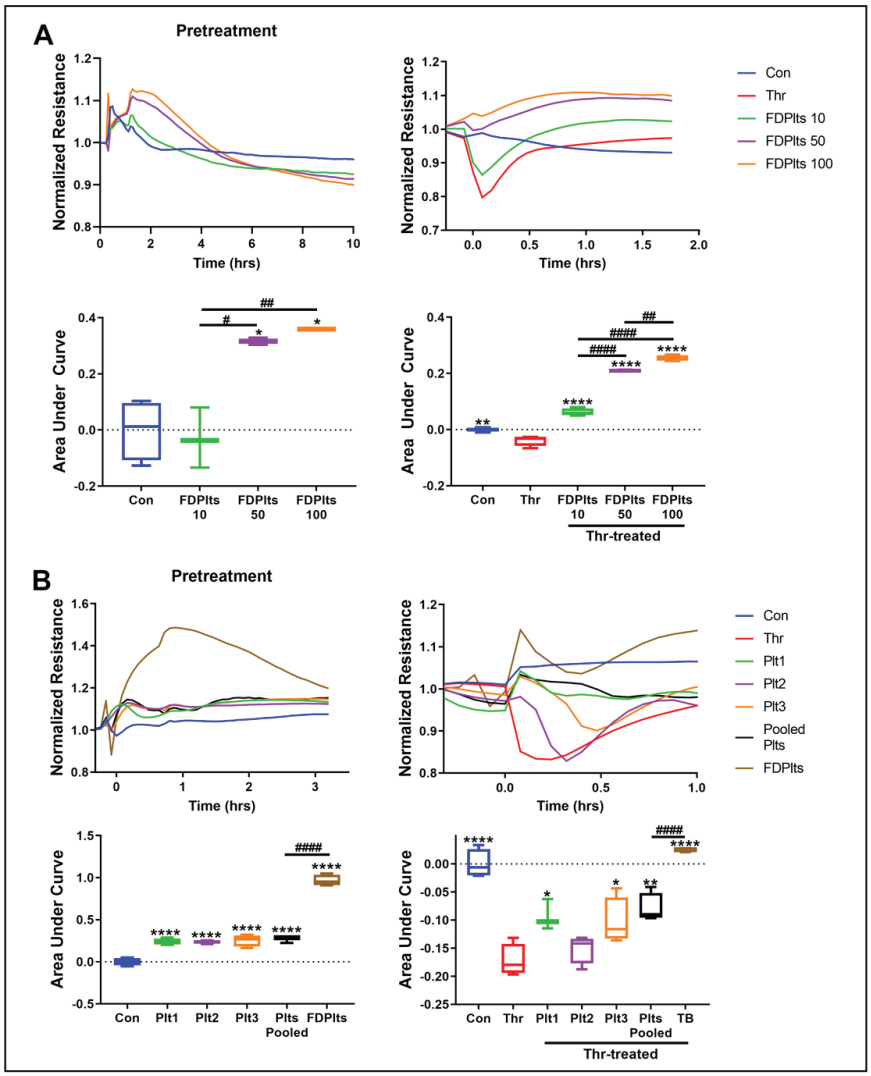


**Figure 2: FDPlts show dose dependent mitigation of thrombin induced endothelial cell permeability.** A) Three doses (10x10^6/ml, 50x10^6/ml, and 100x10^6/ml) of FDPlts were tested in the presence and absence of thrombin challenge on human brain microvascular endothelial cells in ECIS. Top panels show ECIS tracings and bottom panels show quantitation. FDPlts show a dose dependent protection on endothelial cell permeability. B) Fresh apheresis platelets (50x10^6/ml) from 3 different donors and pooled donors were tested in the presence and absence of thrombin challenge on human brain microvascular endothelial cells in ECIS. Donor dependent variability in EC protection is observed, furthermore, FDPlts are more protective than either single donor or pooled donor fresh Plts. One-way ANOVA with Bonferroni post-hoc analysis. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 vs thrombin or control.



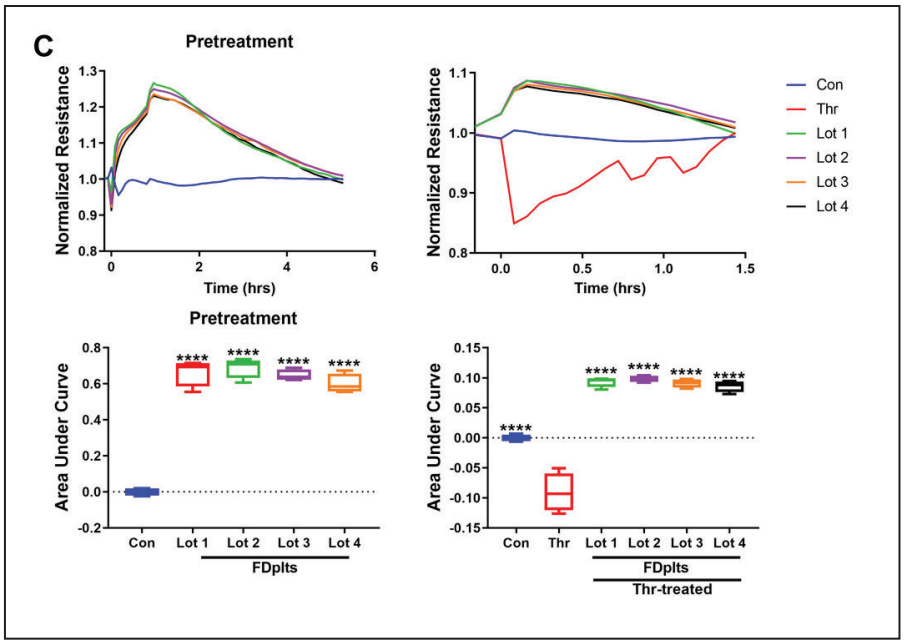



**Figure 3: Consistent mitigation of thrombin induced endothelial cell permeability across multiple FDPlts lots.** Four different lots of FDPlts were tested in the presence and absence of thrombin challenge on human brain microvascular endothelial cells in ECIS. Top panels show ECIS tracings and bottom panels show quantitation. FDPlts show protection on endothelial cell permeability across multiple lots. One-way ANOVA with Bonferroni post-hoc analysis, ****p<0.0001.

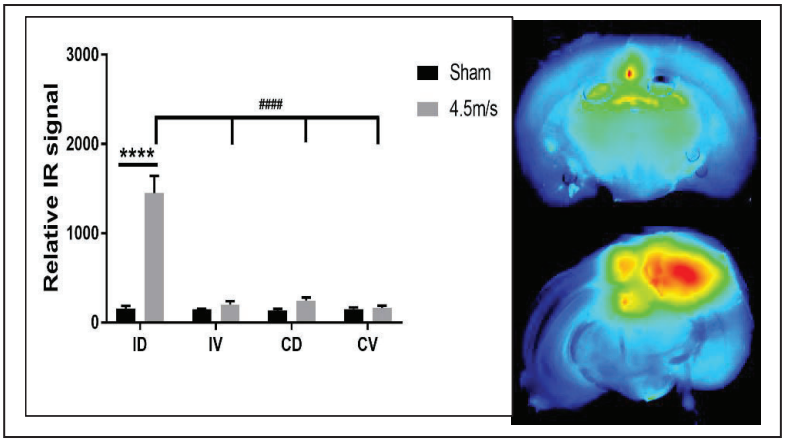
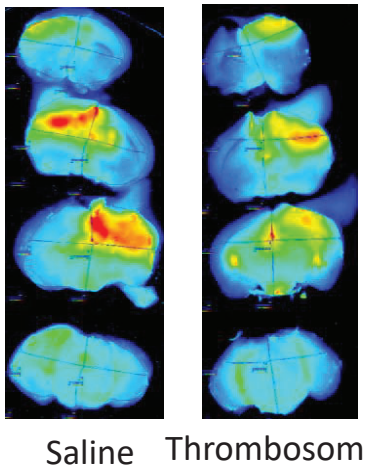
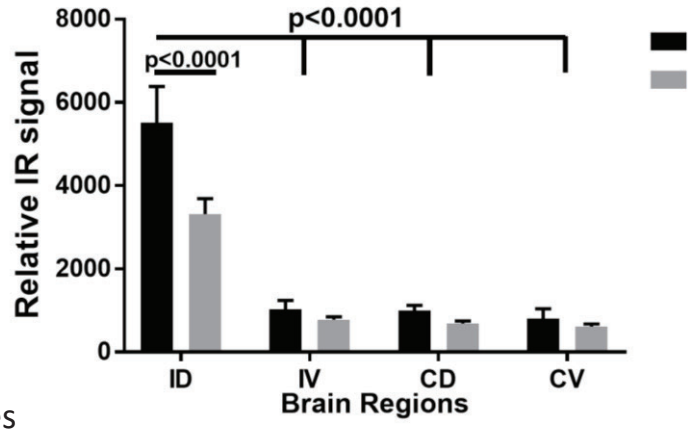



**Figure 4: Traumatic brain injury induced vascular permeability at 3 days post-injury.** Mice were subjected to controlled cortical impact injury or sham surgery (anesthesia and placement on stereotactic device with incision). Permeability was measured by using a 10kD IR-680 tagged dextran. ID- Ipsilateral 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) and sham vs TBI in ID region. ****p<0.0001. n=5-6 per group

**Figure 5: Treatment with FDPlts Attenuates Barrier Disruption at 3 days Post-injury**
Mice were subjected to TBI and treated with either saline or FDPlts 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 FDPlts treated groups show focal increased barrier permeability in the ipsilateral dorsal quadrant as compared to the other three quadrants and the FDPlts treated mice have significantly less disruption as compared to the saline treated mice. Black bars = saline treatment; grey bars = FDPlts treatment; 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) and saline vs. FDPlts in ID region, $p<0.0001$.



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

Nothing to Report

**How were the results disseminated to communities of interest?**

This work on role of FDPlts in mouse model of hemorrhagic shock was presented at the AAST conference (September 2020) and was published in Journal of Trauma and Acute Care (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)
- Start long-term studies in mouse hemorrhagic shock model (Major task 4)
- Continue studies in traumatic brain injury (Major task 5)
- Continue intravital microscopy studies (Major task 6)

## *4.* IMPACT:

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

The protein content of FDPlts is similar to fresh platelets, suggesting that the freeze drying process does not affect the protein content. FDPlts attenuated vascular permeability in vitro in brain microvascular endothelial cells and showed lot to lot consistency unlike fresh platelets that had donor-to-donor variability. FDPlts also attenuated blood brain barrier permeability in a mouse model of TBI. These data suggest that the effect of FDPlts may be a suitable alternative to fresh Plts in modulating endotheliopathy associated with injury.

**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:

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 first manuscript was published in Journal of Trauma and Acute Care (Published article is attached in the appendix).

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

Nothing to Report

**Other publications, conference papers and presentations**.

Nothing to report

- **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 - 2.88
ZHANG,HAOQIAN - 3.92
MIYAZAWA,BYRON YUUJI – 4.39
MAHUVAKAR,ALPA – 4.46

*Name: Shibani Pati MD PhD*
*Project Role: Principal Investigator*
*Nearest person month worked: 2.88 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: 4.46 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: 4.39 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:

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