AWARD NUMBER: W81XWH-19-1-0462

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

PRINCIPAL INVESTIGATOR: Shibani Pati MD PhD

CONTRACTING ORGANIZATION: University of California San Francisco

REPORT DATE: Sept 2020

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Development Command

Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.

1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED *(From - To)*
Sept 2020	Annual	8/15/2020-8/14/2020

4. TITLE AND SUBTITLE

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

5a. CONTRACT NUMBER

W81XWH-19-1-0462

5b. GRANT NUMBER

BA180248

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Shibani Pati MD PhD PI Professor UCSF

Alpa Mahuvakar PhD Researcher UCSF

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

University of California San Francisco
513 Parnassus Avenue HSE760
San Francisco, CA
94113

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

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

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRDC
U	U	U	SAR	22	19b. TELEPHONE NUMBER *(Include area code)*

TABLE OF CONTENTS

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

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

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

3. ACCOMPLISHMENTS: *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official 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: 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	80% 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	50% completed
Subtask 4: Tissue analysis 3 hour HS model (102 animals)	16-18	50% 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	
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	
Subtask 2 - Tissue analysis (barrier permeability – 80 mice)	16-18	
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	
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	
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?

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.

The major work completed during this time was as follows:

1. We wrote and got UCSF IACUC animal protocol approved in October 2019 and ACURO approval in February 2020.
2. We have started in vitro endothelial barrier permeability experiments in endothelial cells using FDPlts (Thrombosomes) and fresh platelets as controls.
3. 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 has been submitted as a manuscript for publication in Journal of Trauma and Acute Care (see Appendix).
4. We have begun traumatic brain injury (TBI) experiments with FDPlts (Thrombosomes) and fresh platelets as controls.
5. Due to space constraints, the behavior room was reestablished at UCSF. We are currently setting up 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.

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.

Intravital microscopy of mouse cremaster vasculature injury model

Adult male C57BL6 mice (8-12 week old) were obtained from Harlan Laboratories (Livermore, CA). Mice were partially depleted of platelets by dosing them with R300 antibody (Emfret analytics) at 0.5µg/g body weight 48 hours before procedure. Mice were anesthetized with an intraperitoneal injection of ketamine (90mg/kg) and xylazine (10mg/kg) and placed on temperature controlled heating pad. The trachea was intubated using polyethylene 90 tubing (Becton Dickinson, Sparks, MD) to allow spontaneous respiration. The left femoral artery was cannulated using Instech mouse femoral cannulas (Instech Laboratories, Plymouth Meeting, PA), for the administration of anesthetic, FITC-dextran, Plts and FDPlts. The cremaster muscle was exteriorized, pinned to the stage and superfused with warm bicarbonate-buffered saline equilibrated with 5% CO_2 in N_2. Using a Leica DM6 FS epifluorescence microscope (Leica, Feasterville, PA) with Hamamatsu camera (Hamamatsu, Bridgewater, NJ) a post capillary venule was located for injury and analysis. A 50µL bolus of 3mg/ml FITC-tagged 70kD dextran in saline was administered via the cannula. A light dye injury in the vessel was induced by shining fluorescence on the vessel, thus provoking clot formation. Vessels were filmed for up to 30 minutes. After baseline measurement of a vessel, TRITC tagged Plts or Alexa Fluor 594 NHS ester tagged FDPlts were administered through the cannula. Once clot formation was complete the time to clot (obstruct blood flow) was recorded and imaged with TRITC illumination revealing the Plts or FDPlts in the clot. Images were captured and analyzed using MetaMorph (Molecular Devices).

Miles Assay of vascular permeability in mice

The modified Miles assay was performed in 8 to 10-week-old NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ (NSG) mice as described previously. Comparison groups were mice treated with saline, Day 1 Plts, or FDPlts at 3 $X10^8$ Plts or particles of FDPlts/mouse, which amounts to approximately 13% of the total Plt count in mice.

Circulation time of FDPlts in naïve mice

$1.5x10^6$ FDPlts or Plts per gram body weight were administered via right femoral artery and blood samples were withdrawn at one, five and ten minutes after administration. Seven animals were analyzed by this method. Flow cytometry was used to assess mouse blood for expression of 1:100 diluted FITC-conjugated anti-CD41 (BioLegend, San Diego, CA), and PE-conjugated anti-CD42b (BioLegend). FDPlts were gated based on size and expression of CD41 and CD42b. All events were analyzed using FlowJo software (FlowJo, Version 9.7, Tree Star, Inc., Ashland, OR).

Hemorrhagic shock and trauma animal model

Male C57BL6 mice, 8-12 weeks old, were obtained from Harlan Laboratories (Livermore, CA) (n = 4-7 per group, total of 29 mice). Under inhaled isoflurane anesthesia, animals were placed on a heating plank to maintain body temperature between 35°C and 37°C. To induce trauma, a 2.5-cm midline laparotomy was performed and then sutured. To generate hemorrhagic shock (HS), femoral arterial catheters were flushed with 1000U/ml heparin and then placed into the femoral arteries of both legs. No additional heparin was used. The left catheter was connected to the corresponding fluid reservoir and the right to a blood pressure monitor (PowerLab 8, AD Instruments, Dunedin, New Zealand). Mice were bled to a mean arterial blood pressure (MAP) of 35 mm Hg for 90 minutes.[17] After the shock period, mice were resuscitated with 1) 200µL LR, 2) $1.5x10^6$ particles/g body weight of FDPlts, 3) $1.5x10^6$ human Plts/g body weight, or 4) shed volume of whole mouse blood (WB) as a positive control for full recovery. Mice were monitored for an additional 30 minutes after resuscitation, before the wounds were sealed. Animals were allowed to ambulate freely with access to food and water for 60 minutes.

Pulmonary vascular permeability analysis

Three hours after the initiation of shock, the animals were anesthetized with 2% isoflurane and 0.2mg/kg of a 10 kDa dextran conjugated with Alexa Fluor 680 was administered via a cannula as previously reported.[18] The left lobe of the lung was dissected out and scanned on the Odyssey Imaging System (Li-Cor scanner, Lincoln, NE), and the infrared signal was read at 700nm. Using image studio software, an average fluorescence signal intensity per lung was quantitated and averaged with others from the same treatment group.

Histologic analysis of tissue

Tissues were prepared and evaluated as previously reported.[18]Sections were imaged at 100x with a Revolve microscope (Echo Inc., San Diego, CA). Two non-serial sections from each mouse were assessed by two blinded researchers before a representative image was selected from each group. Six 100X power fields per mouse were assessed by a scoring system from 1-3 to quantify inflammatory infiltrates and alveolar wall thickening in the lung parenchyma for each group of mice. A score of one was very negligible infiltration, a score of 2 was about 30-40% of area covered with infiltrate and a score of 3 was 60-80% of area covered by infiltrating cells.

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. An α of 0.05 was preset as the cutoff for statistical significance. All data are represented as mean ± SD.

Results: Figure 1 Morphological and functional *in vitro* characterization of FDPlts. (A) SEM shows that FDPlts and activated Plts display an activated state in the form of long, slender, marginal pseudopods (x25,000). (B) Flow cytometry analysis of FDPlts indicated a phenotype of activated platelets with high surface marker expression of CD41, CD62p, and PS (68.0%, 76.6%, and 99.7%, respectively). (C) Qualitative and quantitative evaluation of Plts and FDPlts using Total Thrombus-formation Analysis System (T-TAS) demonstrated adhesion to collagen and thrombus formation under shear flow. Thrombin generation by apheresis Plts and FDPlts were evaluated by the thrombin generation assay (TGA) using Calibrated Automated Thrombogram. Thrombin peak heights were similar between groups.

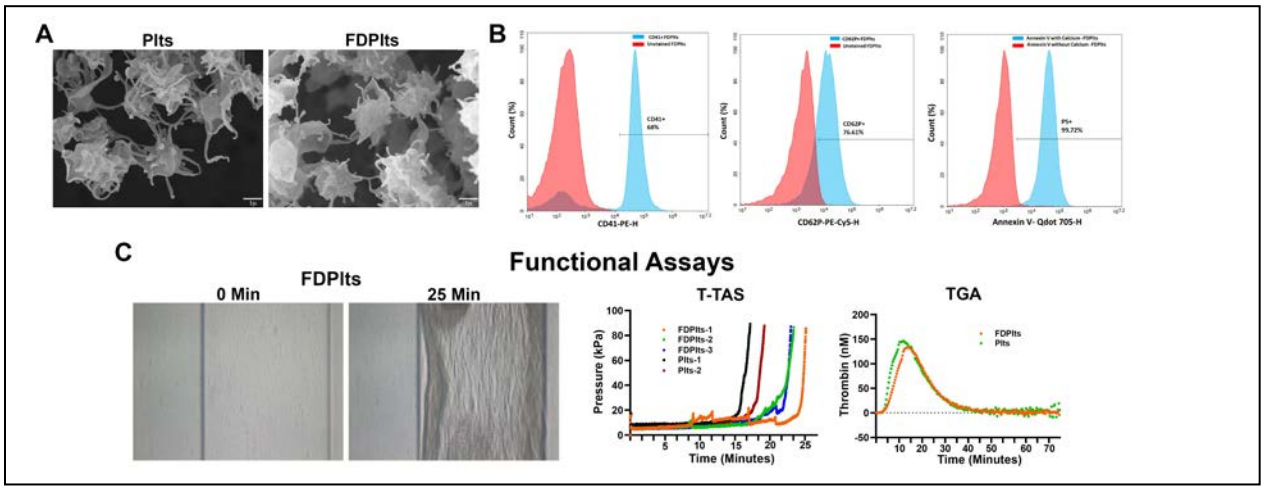


Figure 2: Treatment with Plts and FDPlts protect the endothelial barrier and junctional protein expression *in vitro*. (A) TEER ECIS tracing of human umbilical vein endothelial cells (HUVECs) pretreated with Plts and FDPlts at concentrations of 10x10^6, 25x10^6, 50x10^6, and 100x10^6 particles/ml, at 1hr of treatment. AUC boxplots represent area under the curve quantitation of changes in barrier resistance, compared to control. *Indicates significant difference from control as determined by One-way ANOVA (*p<0.05). Values indicate mean±SD. N=4 wells/condition. Buf is buffer used to reconstitute FDPlts.

(B) TEER tracing of HUVECs challenged with thrombin (0.2U/ml) following Plts and FDPlts treatment. AUC boxplots represent area under the curve quantitation of changes in barrier resistance, compared to control. *Indicates a significant difference from thrombin, as determined by One-way ANOVA (****p<0.0001). Values indicate mean±SD. N=4 wells/condition (C, D) Representative images of HUVECs treated with Plts or FDPlts ($50x10^6$ particles/ml) and subsequently challenged with thrombin (0.2U/ml), stained for VE-cadherin, f-actin, and ZO-1, with gap lengths between cells quantified. * Indicates different from thrombin, as determined by One-way ANOVA, where *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001. Columns indicate mean±SD. N=3 coverslips/treatment and 3 images per coverslip

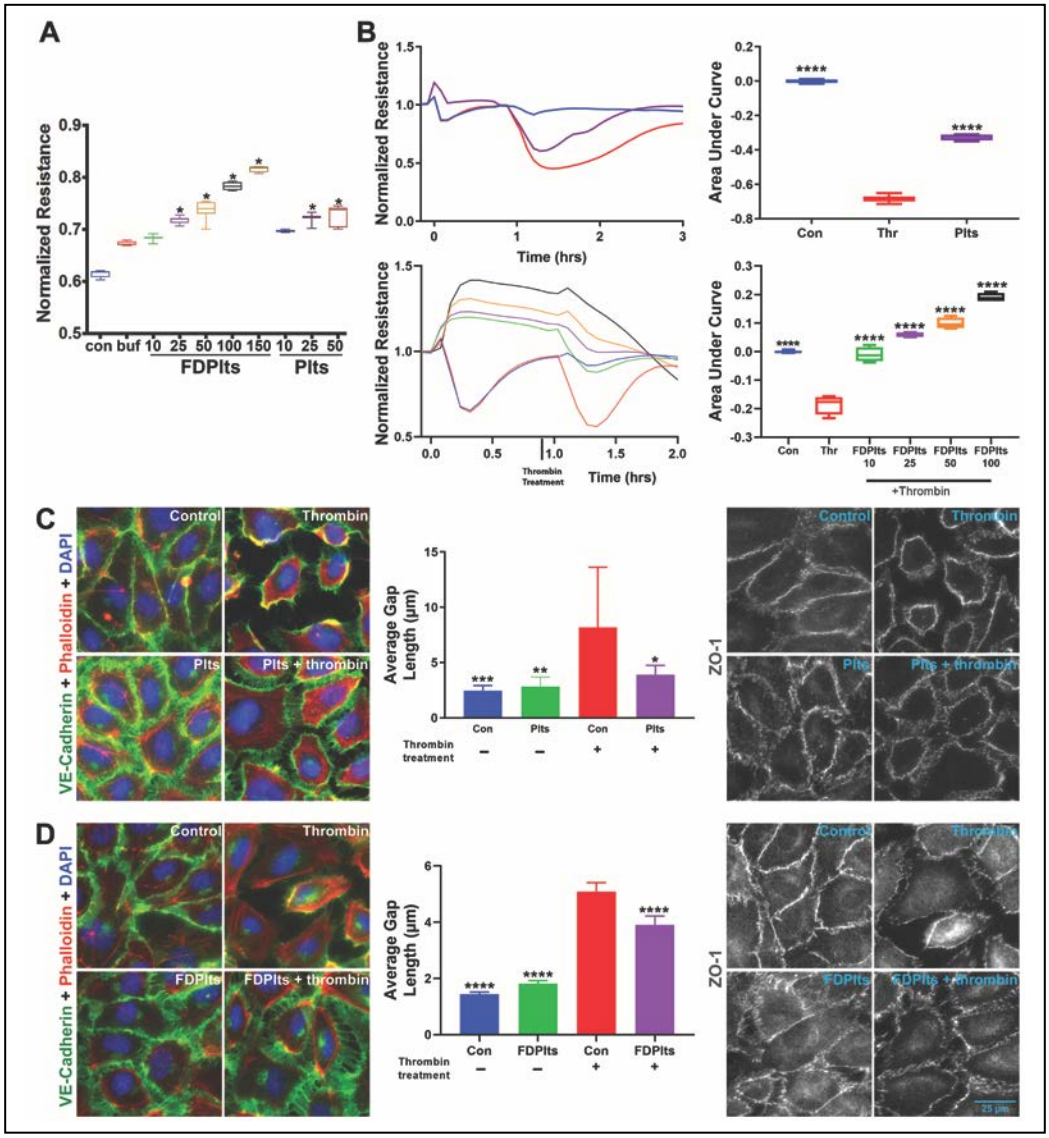


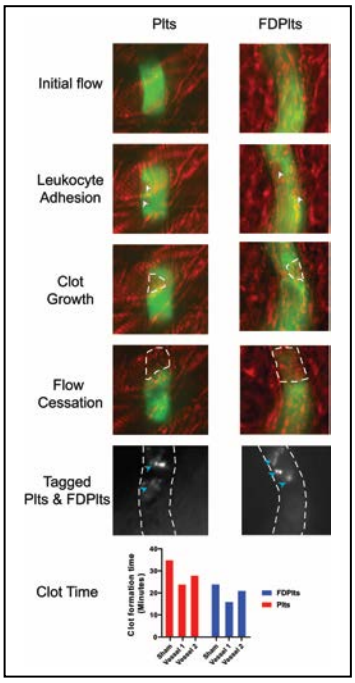
Table 1: Aggregation of FDPlts was evaluated at a concentration of 375K/µl in response to thrombin and ranged from 65 to 53% in comparison to washed apheresis Plts (Day 1-3) at 250K/µl ranging from 95 to 97%.

Aggregation to Thrombin

FDPlts @ 375k/µl		Washed Apheresis Plt @ 250k/µl*	
Lot	**Result**	**Lot**	**Result**
E-T-19-013	62%	E-T-18-048	95%
E-T-19-016	65%	E-T-18-052	98%
E-T-19-017	53%	E-T-18-056	97%
Mean/StDev	**60%±5.10%**		**97%±1.27%**

*Aggregation to thrombin was discontinued as a quality control test of apheresis platelets in 2019. The data from 2018 are provided and are typical of results expected from platelets collected by apheresis. Function and activation state of platelets in vitro depend on apheresis modality. S. Macher, S. Sipurzynski-Budraß, K. Rosskopf, E. Rohde, A. Griesbacher, A. Groselj-Strele, G. Lanzer, & K. Schallmoser.; Vox Sanguinis (2010) 99, 332–340

Figure 3: Both Plts and FDPlts are incorporated into clots in a mice and decreased clot formation time in a platelet depleted mouse. Representational images of clot formation and visualization of (red) Plts and FDPlts incorporated in the clots. Graphical representation of clot formation time after fluorescence induced injury before and after FDPlts or Plts treatment.



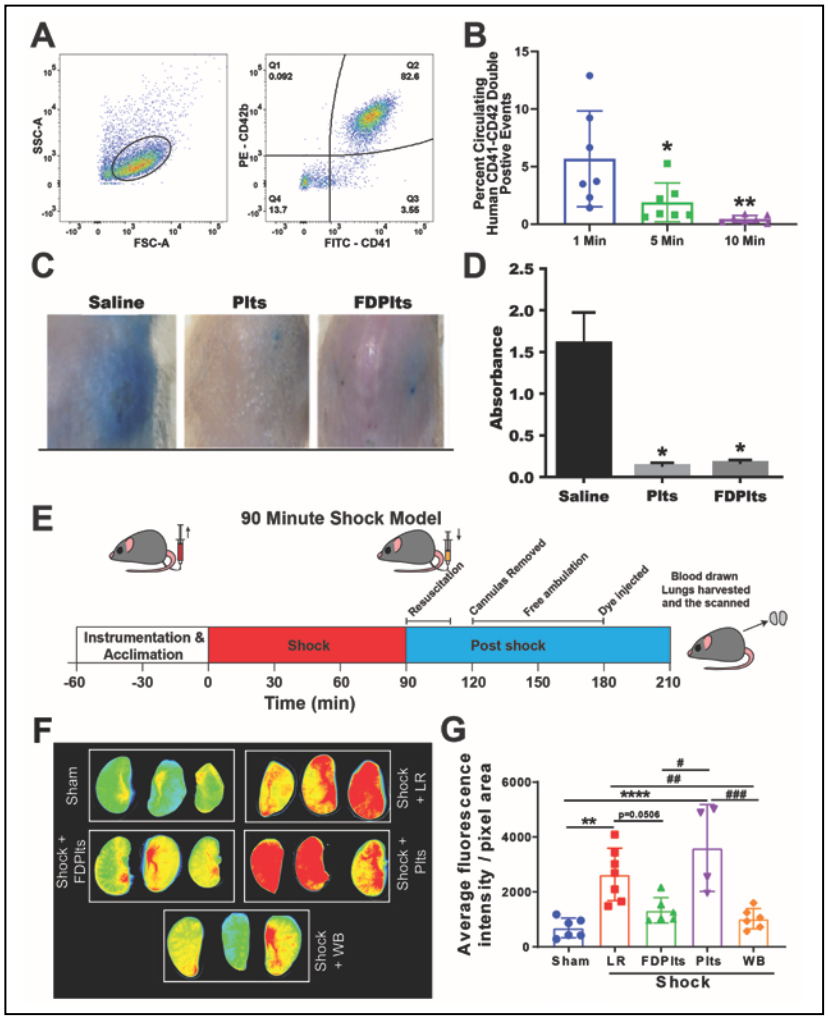
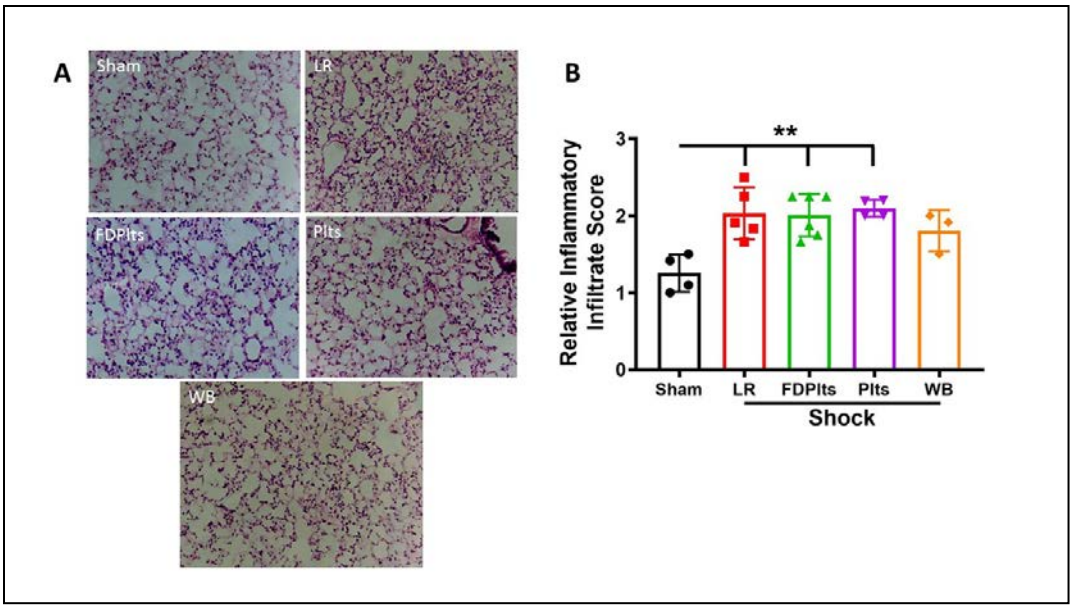


Figure 4: FDPlts restore VEGF-A induced dermal vascular permeability and pulmonary barrier function in mice subjected to HS

Flow cytometry analysis of circulating FDPlts. Events were gated based on forward and side scatter to identify platelets by size. This gated population was identified as FDPlts based on co-expression of human CD41 and CD42b. Quantitation of numbers of FDPlts showed a decrease in circulating CD41 and CD42b positive events over time as determined by One-way ANOVA followed by Tukey's multiple comparisons test; *p< 0.05; **p < 0.01. Columns and error bars indicate mean ± SD. (B) Quantification of Evans Blue extravasation from mice treated with saline, Day1 Plts, or FDPlts and representative images of vascular leak of Evan's Blue dye in each of the groups. (C) Schematic of HS experiment (D) Representative images of the scanned left lobes of mouse lungs to detect vascular permeability (left panel). Quantitation of fluorescence intensity of Alexafluor 680 conjugated 10 kDa dextran in the left lobes of the mouse lungs. Shock and treatment with LR induces permeability. This is attenuated by treatment with FDPlts and WB but not by Plts treatment (right panel). One-way ANOVA followed by Tukey's multiple comparisons test; *p< 0.05; **p < 0.01; ***p<0.001; ****p<0.0001. Columns and error bars indicate mean ± SD. N=4-7 mice/treatment group.

Figure 5: Histopathological analysis of lung sections at 3 hours post-shock displays increased inflammatory cell infiltrate Representative images of lung sections stained with H&E for the 5 mouse groups: sham, shock+LR, shock+Plts, shock+FDPlts, and shock+Whole Blood (WB). Significant differences in inflammatory infiltrates are found between the sham and shock+LR, shock+FDPlt and shock+Plt treated mouse lungs are evident at 3 hours after initiation of shock. However, there are no significant differences between sham and shock+WB mice at this time point. One-way ANOVA followed by Tukey's multiple comparisons test; **p < 0.01; Columns and error bars indicate mean ± SD. N=4-7 mice/treatment group. 100X magnification



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.

Lindsay Vivona, our technician in the laboratory would like to go to graduate school. We have trained her with techniques such as tissue processing, staining and analysis abilities including microscopy and flow cytometry. She has also co-authored the paper due to her contribution to the study.

Alpa Mahuvakar was able to present her preliminary work on Thrombosomes in traumatic brain injury at the CTTAC meeting, December 2019.

Byron Miyazawa has learnt techniques such as Western blotting, lysate preparation, and multiplex ELISA.

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.

The work on FDPlts and traumatic brain injury has been presented at CTTAC, December 2019 (see Appendix).
This work on role of FDPlts in mouse model of hemorrhagic shock was presented at the AAST conference (September 2020) and has been submitted as a manuscript for publication in Journal of Trauma and Acute Care (see Appendix).

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.

- 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).
- Set up behavior room (Major task 5, subtask 6)
- Start short-term dose response studies in mouse hemorrhagic shock model (Major task 3, subtask 2)
- Start long-term studies in mouse hemorrhagic shock model (Major task 4)
- Start studies in traumatic brain injury (Major task 5)
- Continue intravital microscopy studies (Major task 6)

4. **IMPACT:** D*escribe 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).

Our data suggests that FDPlts contribute to clot formation similar to fresh human Plts. FDPlts also attenuated vascular permeability in vitro. FDPlts attenuated vascular permeability after hemorrhagic shock at levels comparable to mouse whole blood resuscitation but fresh human Plts did not have a similar effect. These data suggest that the effect of FDPlts may be a suitable alternative to fresh Plts in modulating hemostasis and the endotheliopathy associated with injury. This supports the use of FDPlts in clinical practice due to the advantage of storage and preserved functionality.

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.

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?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:
- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to report.

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

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

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

Nothing to Report

5. CHANGES/PROBLEMS: *The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official 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:*

Nothing to Report

Remember that significant changes in objectives and scope require prior approval of the agency.

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.

1. Due to space constraints, the behavior room was reestablished at UCSF. We are currently setting up behavior room in the barrier facility with new equipment and will set up all tests. 2. Due to COVID-19 we were give work from home orders for 2 months. During this time, we worked on the manuscript and put together data for the submission. Now we are at partial capacity (25%) but have been cognizant of our commitment and deliverables and as such we have been able to accomplish the proposed tasks.

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.

Nothing to Report

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

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: *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).*

We have submitted our first manuscript to Journal of Trauma and Acute Care (submitted manuscript is attached in the appendix).

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

Nothing to Report

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

This work was presented at CTTACC 2019, December 11-13, San Diego, CA (Talk is attached as pdf in Appendix) and AAST 79th Annual Meeting, September 2020.

- **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

- *Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were 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. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

Nothing to Report

- **Other Products**
  Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding,

prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:

- *data or databases;*
- *physical 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".

Example:

Name:	*Mary Smith*
Project Role:	*Graduate Student*
Researcher Identifier (e.g. ORCID ID):	*1234567*
Nearest person month worked:	*5*
Contribution to Project:	*Ms. Smith has performed work in the area of combined error-control and constrained coding.*
Funding Support:	*The Ford Foundation (Complete only if the funding support is provided from other than this award.)*

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?
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.

None

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.*

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

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: *For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to* ***https://ers.amedd.army.mil*** *for each unique award.*

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

9. APPENDICES: *Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.*

CTTAC December 2019

Alpa Mahuvakar Trivedi - Freeze Dried

JOTAC submitted manuscript

Trivedi et al.pdf