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14. ABSTRACT

Combat-associated traumatic hemorrhage remains a primary cause of 'preventable mortality' in the military. As evidenced by robust clinical studies and dictated by current Remote Damage Control Resuscitation (RDCR) principles, timely transfusion of blood components (platelets, RBC, plasma) to mitigate hemorrhagic shock strongly improves combat casualty survival. However, blood components, especially platelets, have very limited availability far forward and there is a severe lack of *field-deployable* platelet products to enable effective *in-field* Hemostatic Resuscitation (HR). Lyophilized, low-volume, portable, easily storable, saline-reconstitutable synthetic platelet surrogate technology can potentially address this significant challenge and improve survival outcomes. To this end, we have developed a liposome-templated synthetic platelet surrogate technology (**SynthoPlate (SP)**, ***US 9107845*** and ***US 93636383***, **TRL 4**) that has demonstrated systemic safety, targeted hemostatic efficacy and survival improvement in pilot studies in mouse, rat and pig hemorrhagic trauma models. We have established the ability to lyophilize and reconstitute SynthoPlate particles, as well as the ability to sterilize them for long term storage (12 months), without compromising their stability and platelet-mimetic bioactivity. Building on these successful capabilities, we propose the translational development of the 'lyophilized SynthoPlate' (**Lyo-SP**) nanotechnology as an intravenous or intraosseous administrable platelet surrogate product with a vision for RDCR application in combat-associated trauma for point-of-injury 'hemorrhage control' and TIC management.

15. SUBJECT TERMS

NONE LISTED

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TABLE OF CONTENTS

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

Combat-associated traumatic hemorrhage remains a primary cause of 'preventable mortality' in the military. As evidenced by robust clinical studies and dictated by current Remote Damage Control Resuscitation (RDCR) principles, timely transfusion of blood components (platelets, RBC, plasma) to mitigate hemorrhagic shock strongly improves combat casualty survival. However, blood components, especially platelets, have very limited availability far forward and there is a severe lack of *field-deployable* platelet products to enable effective *in-field* Hemostatic Resuscitation (HR). Lyophilized, low-volume, portable, easily storable, saline-reconstitutable synthetic platelet surrogate technology can potentially address this significant challenge and improve survival outcomes. To this end, we have developed a liposome-templated synthetic platelet surrogate technology (**SynthoPlate (SP)**, ***US 9107845*** and ***US 93636383***, **TRL 4**) that has demonstrated systemic safety, targeted hemostatic efficacy and survival improvement in pilot studies in mouse, rat and pig hemorrhagic trauma models. Recently we have established the ability to lyophilize and reconstitute SynthoPlate particles, as well as the ability to sterilize them for long term storage (6-9 months), without compromising their stability and platelet-mimetic bioactivity. Building on these successful capabilities, we propose the translational development of the 'lyophilized SynthoPlate' (**Lyo-SP**) nanotechnology as an intravenous or intraosseous administrable platelet surrogate product for point-of-injury 'hemorrhage control' with a vision for RDCR application in combat-associated trauma to achieve hemorrhage control and treat trauma-induced coagulopathies.

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

Hemorrhage, Trauma, Coagulopathy, Platelets, Synthetic Platelets, Field-deployable, Lyophilizable, Intravenous, Intraosseous, Remote Damage Control Resuscitation

3. **Accomplishments:** The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction.

What were the major goals of the project?
List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project identify these dates and show actual completion dates or the percentage of completion.

The overall Specific Aims of the PR191632 project as approved in the original SOW are:

Specific Aim 1: In vitro evaluation of platelet-relevant bioactivity for Lyo-SP compared to freshly made SP, utilizing microfluidics, aggregometry, flow cytometry and ROTEM using human (healthy donor and trauma patient) plasma and whole blood.

Specific Aim 2: In vivo evaluation of systemic safety, hemostatic efficacy and survival outcome for Lyo-SP compared to freshly made SP, varying dosage and time-of-administration in rat liver resection lethal hemorrhage model.

Specific Aim 3: In vivo evaluation of systemic safety, method and time of delivery, hemostatic efficacy and survival outcome for Lyo-SP compared to freshly made SP, in pig intraperitoneal hemorrhage model.

The project was officially funded in July of 2020, because of COVID-19 related restrictions and shutdowns in 2020 and first half of 2021, the purchasing and research operations could be technically resumed only in late Spring of 2021. **To that end, we have now an approved 12-month NCE.** The current report is the annual report of July 2022- Aug 2023.

Also, the **Aim 3** studies were proposed to be conducted in a pig model of intraperitoneal hemorrhage. However, due to a variety of specific physiological issues that are present in cloven-hoofed animals (e.g. pig, sheep) that make them uniquely sensitive to nanoparticle systems in cardiopulmonary circulation, a model change was requested from pigs to rabbits. Specifically, pigs are hypersensitive and uniquely reactive to *intravenously* administered nanoparticle systems and show transient signs of cardiopulmonary distress, which ultimately resolve via modulation of dose infusion rate and nanoparticle surface charge, but nonetheless puts a complex operational obstacle in doing pig model studies. In our literature search on this matter, we found that this observation has been documented by several other researchers in studying other nanoparticle systems (e.g. hemoglobin-loaded nanoparticles as RBC surrogates, chemotherapy-loaded liposomal nanoparticles etc.) intravenously administered in pigs, and the current consensus is that this is attributed to a special type of macrophages (pulmonary intravascular macrophages, PIMs) that are present in pigs and other cloven-hoofed animals, but not in humans (and thus the human exhibition of such reactions to I.V.-administered nanoparticles is rare). We therefore rationalized that for our non-rodent studies **we should choose a non-cloven hoofed animal**, and based on reported DOD studies from other research groups we selected rabbits as a suitable non-rodent model (based on robust documentation of blood product evaluation by DOD-funded investigators in rabbit trauma models). Therefore, the revised Specific Aim 3 is:

Specific Aim 3: Evaluation of systemic safety, method and time of delivery, hemostatic efficacy and survival outcome for reconstituted Lyo-SP compared to Liq- SP, **in rabbit intraperitoneal polytrauma hemorrhage model.**

What was accomplished under these goals?

For this quarterly reporting period only 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.

Under **Specific Aim 1**, the following were accomplished during this year:

Major Task 1: Process establishment for reproducible manufacturing, lyophilization, sterilization and rapid small volume aqueous reconstitution of Lyo-SP.

This task was completed by collaboration between Haima Therapeutics (Bruckman, Co-I), in establishing and validating the process for lyophilized SynthoPlate (Lyo-SP) under the formulation name HT-12. Shown below are representative final data of HT-12 Lyo-SP:

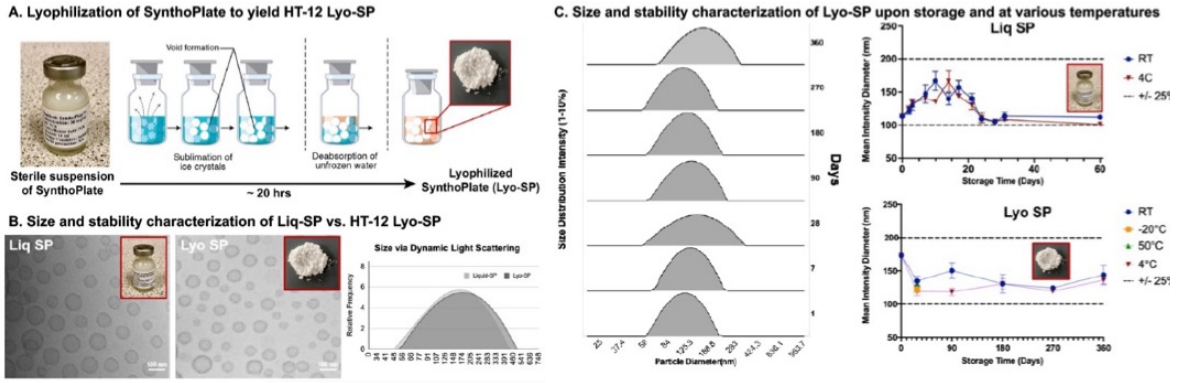

Figure 1. Manufacture and characterization of Lyo-SP. A: Lyophilization process developed by Haima Therapeutics with proprietary lyoprotectant formulation enables efficient manufacture of Lyo-SP from Liq-SP; **B:** Dynamic Light Scattering (DLS) and cryo-Transmission Electron Microscopy (cryo-TEM) studies confirm that lyophilization of SP and subsequent aqueous reconstitution of Lyo-SP conserves the vesicular morphology and size (100-200 nm diameter) of the particles; C: The size distribution of room-temperature (RT) stored Lyo-SP is conserved over at least a 1-year period, indicating its morphological stability in long-term storage; Additionally, the size (hence stability) of Liq-SP is maintained at RT and 4°C, while Lyo-SP size (hence stability) is maintained further over a range of -20°C to 50°C.

Major Task 2: Establishment of particle stability, morphology, and platelet-mimetic bioactivity of Lyo-SP utilizing microfluidics, aggregometry, flow cytometry, viscoelastometry analysis with healthy human donor blood.

This task was completed via collaboration between Haima Therapeutics (Bruckman, Co-I) and Sen Gupta (PI) laboratories. For all these studies the HT-12 Lyo-SP formulation was used. Shown below are representative final data for this task:

- Particle stability and morphology were established by the manufacturing collaborator (Haima) as shown by the results of **Figure 1** on the previous page.

- Platelet mimetic bioactivity using BioFlux microfluidics are shown below in **Figure 2**:

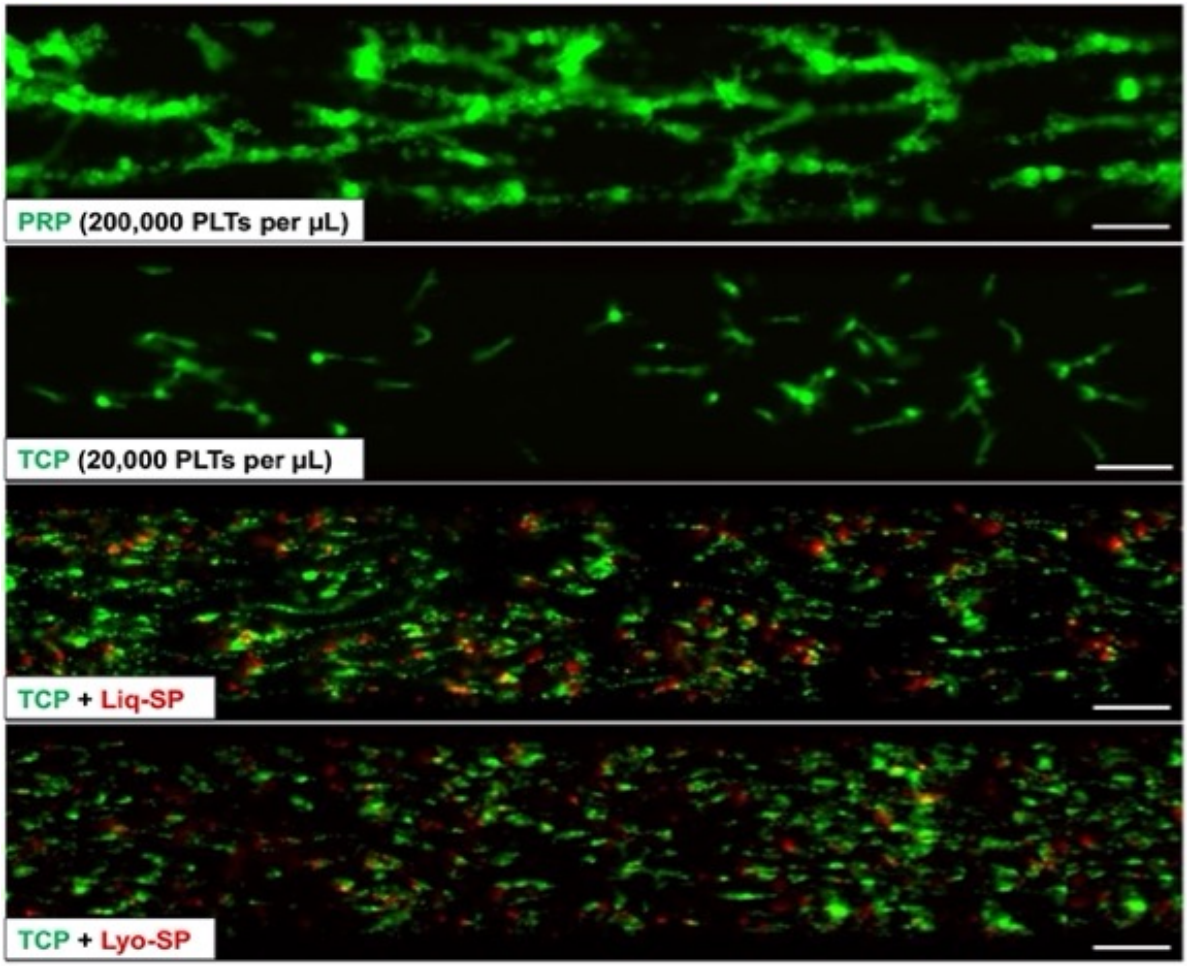

Figure 2: Plasma containing Calcein-stained (green) platelets were flowed over collagen-coated microfluidic channels in presence of soluble VWF at 60 dyn/cm^2 shear flow; Healthy platelet-rich plasma (PRP, 200K platelets/µL) showed significant coverage on the channel (reflective of high platelet adhesion ad aggregation), but this surface-coverage was significantly reduced when platelet numbers were significantly depleted (thrombocytopenic plasma, TCP, 20K platelets/µL); Fresh-made liquid SynthoPlate (Liq-SP) and aqueous-reconstituted Lyo-SP (both red fluorescent) were both able to substantially rescue the channel surface coverage of platelets in TCP condition, indicating the equal capability of Liq-SP and Lyo-SP to mimic and enhance platelet adhesion and aggregation mechanisms.

- Representative aggregometry and ROTEM (NATEM) data are shown in **Figure 3**:

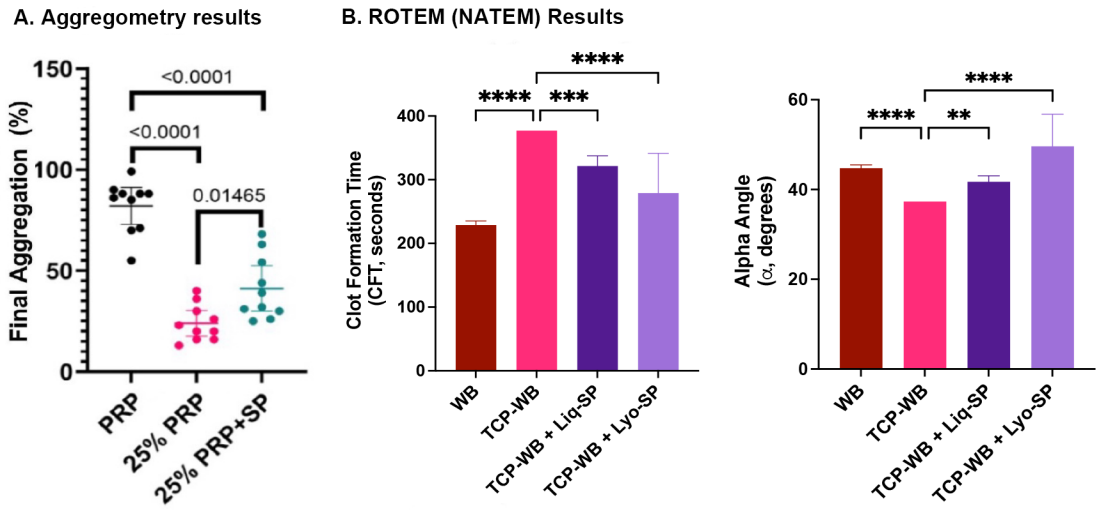

Figure 3. A: In light transmission aggregometry (LTA) platelet aggregation was significantly reduced when PRP was 75% diluted with platelet-free plasma (shown here as 25% PRP) and treatment with SP was able to partly rescue the platelet aggregation outcome, although not to the level of PRP; **B:** In ROTEM (NATEM) analysis, whole blood (WB) was depleted of platelets to create thrombocytopenic whole blood (TCP-WB) and this showed a significant delay in clot formation time (CFT) and alpha angle in ROTEM indicating compromised clotting kinetics; Treatment of TCP-WB with Liq-SP or Lyo-SP rescued these parameters.

- Flow cytometry analysis of SP binding to activated platelets shown in **Figure 4:**

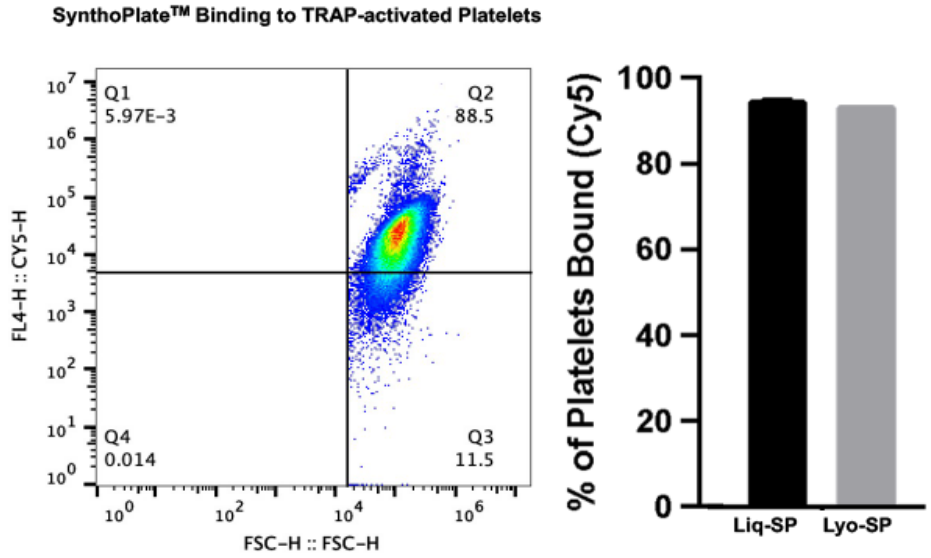

Figure 4. The fibrinogen-mimetic peptide (FMP) decoration on SP is designed to bind to integrin GPIIb-IIIa on activated platelets; Cy5-labeled Liq-SP and Lyo-SP were both able to significantly bind to TRAP-6 activated platelets at comparable extent, indicating that lyophilization and reconstitution of SP does not compromise FMP bioactivity.

Major Task 3: Establishment of clinical feasibility of Lyo-SP hemostatic activity via evaluation with whole blood and plasma derived from human trauma patients.
These studies were initiated in the Fall of 2022 (HRPO was approved in June 2022) and are currently ongoing. Described below are methodologies and representative data:

<u>TEG and Aggregometry Methods</u>: Trauma patient blood samples were collected (Pitt IRB#19080136, HRPO Log Number E01542.2a) based on the following inclusion/exclusion criteria. Red blood cell count, platelet count, hematocrit and hemoglobin were determined using freshly sampled blood collected in EDTA vacutainers (BD Vacutainer, Catalog #367841) using the ADVIA 2120i hematology system (Siemens Medical Solutions USA, Inc., Malvern, PA, USA). Thromboelastography measurements were obtained using a TEG 6S system. A citrated: K, KH, RT and FF cartridge from Haemonetics was used (Haemonetics, Catalog # 07-601-US). Briefly, whole blood (WB) from trauma patients was collected in citrated tubes (BD Vacutainer, catalog #363083). Within 2 hours, 8 uL of treatment conditions was added to 400 uL of trauma patient blood samples. Vehicle (buffer), control particle (CP), fresh-made liquid SynthoPlate (SP), or aqueous-reconstituted lyophilized SynthoPlate (Lyo-SP) was added to trauma blood samples at a concentration of 1:1000, assuming a conserved platelet count of 200 x10^3cells/µL. Additionally, whole blood impedance aggregometry (Chronolog Model 700 Aggregometer) was run on trauma patient samples. Vehicle, control particle (CP), fresh-made liquid SynthoPlate (SP), or aqueous-reconstituted lyophilized SynthoPlate (Lyo-SP) was added to trauma blood samples at a dilution of 1:100, assuming a conserved platelet count of 200 x10^3cells/ µL. Samples were dosed with treatment groups right before starting the assay. Fibrillar Type I Collagen (CHRONO-LOG Corp, Catalog # 385) at a concentration of 2ug/mL was used as an agonist. Assay was run for 6 minutes after adding the agonist. Representative patient data are shown in **Figure 5** and **Figure 6**:

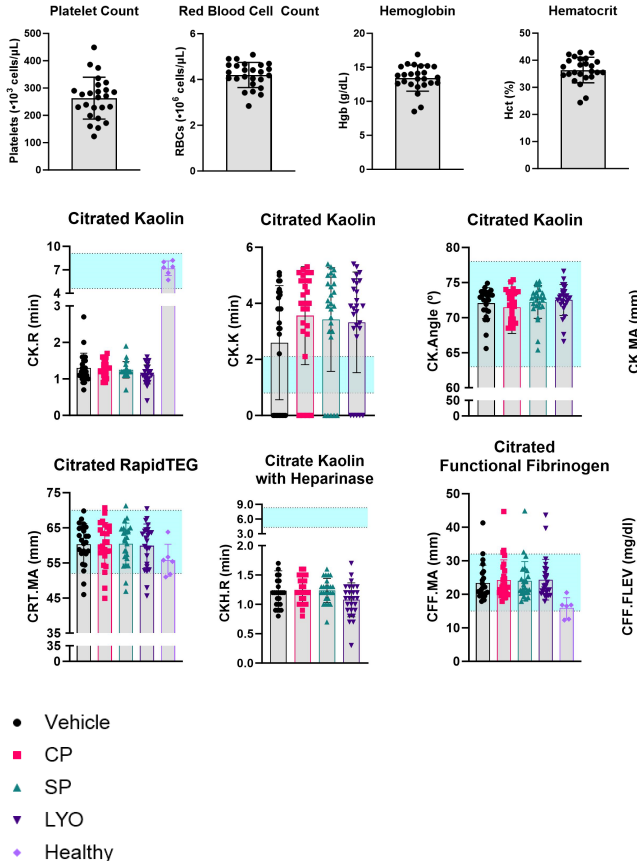

Figure 5. Patient blood count data prior to ex vivo studies with Liq-SP or Lyo-SP.

Figure 3: TEG data. **CK:** Citrated Kaolin (Intrinsic Pathway)**, CRT**: Citrated RapidTEG (Intrinsic + Extrinsic Pathway), **CKH:** Citrated Kaolin with Heparinase (Monitors the effect of Heparin), and **CFF**: Citrated Functional Fibrinogen (Blocking Platelet Contribution to clotting). **R**: Clotting Time, **K**: Clot Formation Time, **Angle:** Rate of Clot Formation, **MA:** Maximum Clot Strength, **FLEV:** Concentration of Functional Fibrinogen. Blue band indicates 'normal' reference range.

From the above data, it is apparent that the largest 'defect' in current cohort of patients is clotting time and clot formation time, and in the current set of data there is no conclusive indication of an 'effect' (positive or negative) of Liq-SP or Lyo-SP in these studies. We will continue these studies with varying SP dose, as well as segmenting the data for Level 1 vs Level 2 trauma patient categories to explore whether an effect of SP emerges.

Impedance aggregometry data is shown in **Figure 7**:

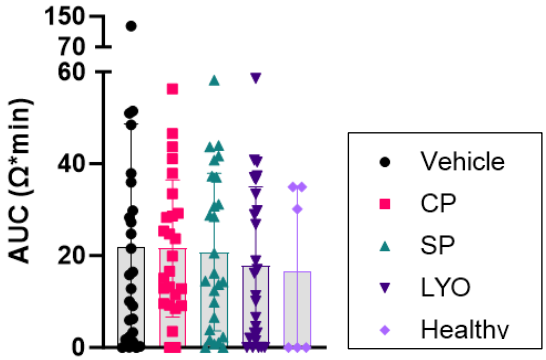

Figure 7. Impedance aggregometry data indicates high variability in aggregation profile of platelets in the blood of both healthy volunteers and trauma patients; Ongoing studies are exploring the reason behind this high variability, since this needs to be resolved to enable analysis of SP effect in such patients.

Microfluidic Methods: A custom-made polydimethylsiloxane (PDMS) microfluidic device was designed to evaluate platelet function ex vivo. Briefly, micromachined molds were fabricated to produce channels 142 um x 460 um (height x width), with a stenosed (narrowed) center 46 um x 460 um (height x width). PDMS (Sylgard 184 Silicone Elastomer Base and curing agent at a ratio 10:1) cured in the mold was removed, inlet and outlet openings created with 1.5mm biopsy punch, and PDMS was then plasma bonded using a benchtop plasma cleaner (Harrick Plasma) to a microscope slide. Channels of bonded chambers were sterilized with 70% ethanol, dried, and coated with fibrillar type I collagen for 1 hour (CHRONO-LOG Corp, Catalog # 385) diluted with 0.9% sodium chloride (NaCl) (5:1 NaCl:Collagen). Channels were then rinsed with phosphate buffered saline (PBS). Whole blood (WB) from trauma patients was collected in heparinized tubes (BD Vacutainer 367874). For visualization, blood samples were incubated with Human TrueStain Fc receptor blocking solution (Biolegend 422302) and fluorescent anti-CD41 (Novus Biological, catalog #NB100-2614JF549). Following staining, blood was filtered through a 40 µm filter prior to perfusion into the microfluidic device. Cy5 stained treatment groups including vehicle, control particle (CP), synthoplate (SP), or lyophilized synthoplate (lyo-SP) was added to trauma blood samples at a dilution of 1:1000, assuming a conserved platelet count of 200 $x10^3$cells/uL. The microfluidic channel inlet was connected to a syringe pump (Harvard Apparatus) for perfusion of WB at an initial wall shear rate at the stenosis of 3500 s-1. A pressure transducer was positioned upstream from the stenosis channel for pressure monitoring during thrombus formation. Experiments were run for a duration of 10 minutes. Images were collected using an inverted microscope (Axio Observer, Zeiss; 5x magnification; 1 frame per 7 seconds) at the throat of the stenosis for the duration of the experiment to visualize thrombus formation in real time. Shown in Figure 8 below is the schematic representation of this high throughput microfluidic system:

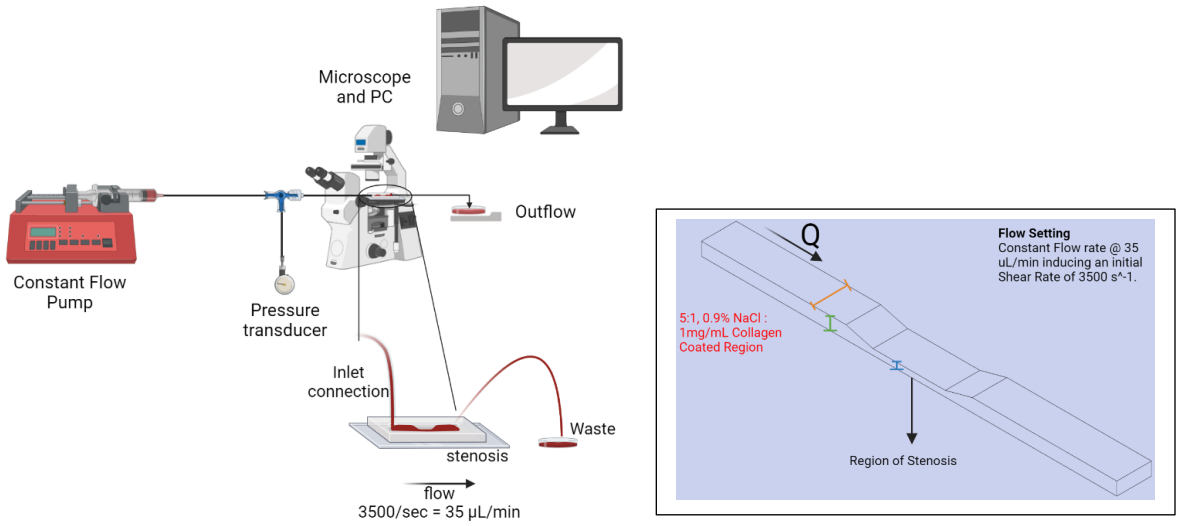

Figure 8: Microfluidic schematic depicts syringe pump and pressure transducer set up upstream of microfluidic device situated on a microscope stage; For the duration of the experiment, images were collected, and pressure was recorded in real time during clot formation; A magnified view of the 'stenosis' microfluidic channel design is shown where Q indicates flow direction; Clot forms at the stenosis region where the shear rate is 3500 s^{-1}.

Representative results from the ongoing studies are shown below in **Figure 9**:

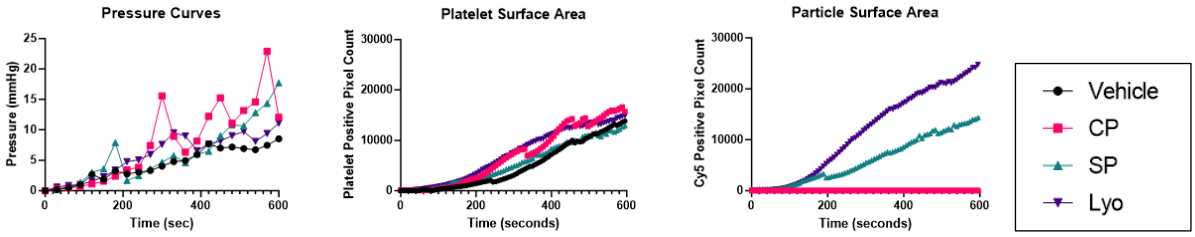

Figure 9: Microfluidic data. **Left)** Pressure change per 30 seconds during 10 minutes of perfusion. **Middle)** Recipient platelet surface area per approximately 7 seconds during 10 minutes of perfusion. **Right)** Particle Surface area per approximately 7 seconds during 10 minutes of perfusion, Vehicle treatment group does not have particle surface area as it was not stained Cy5 and contained no particles; Data indicates significant bioactivity and co-aggregation of Liq-SP and Lyo-SP (but not CP) with platelets in forming the clots in the stenosis channel.

Under **Specific Aim 2**, the major tasks executed during this year are:

<u>Major Task 1:</u> Evaluation of systemic safety, biodistribution and maximum tolerated dose (MTD) of SP versus Lyo-SP in uninjured rats.

Subtask 1: IACUC and DoD ACURO approval for rat studies protocol.

This subtask was completed as IACUC and ACURO approvals for both intravenous studies in rats (at CWRU, ACURO protocol PR191632.e001) and intraosseous studies in rats (at UPitt, ACURO protocol PR191632.e002) were obtained.

Subtask 2: Carry out safety, biodistribution and dose studies in uninjured rats dosed intravenously with Liq-SP or Lyo-SP

- Manufacture and sterilize Liq-SP and Lyo-SP by protocol established in Sp Aim 1
- Carry out safety, biodistribution and MTD studies via Liq-SP or Lyo-SP administration through tail vein in uninjured rats
- Carry out safety, biodistribution and MTD studies via Liq-SP or Lyo-SP administration intraosseously in uninjured rats

SynthoPlate (SP, fresh-made Liq-SP or lyophilized Lyo-SP) were manufactured for these studies by Haima Therapeutics (Bruckman, Co-I) and supplied to Sen Gupta laboratory at CWRU (for intravenous studies in rats) and Neal laboratory at UPitt (for intraosseous studies). The safety, biodistribution and MTD studies with I.V.-administered SP at CWRU were initiated and are currently ongoing. Representative study description and results are as follows:

Methods for safety studies for I.V.-administered SP: Cy-5-labeled Liq-SP, Lyo-SP, and vehicle (buffer) control were manufactured and provided by Haima to Sen Gupta laboratory at CWRU. Sprague-Dawley rats (~ 250-350 g) were housed in the Animal Research Center at CWRU and acclimated to the space for 48 hours. Rats were weighed to inform the dosing amount and then anesthetized with 2-4% isoflurane. Anesthesia was confirmed via toe pinch before being transferred to a nose cone. Ophthalmic ointment was applied to prevent the eyes from drying out. Rectal thermometer, pulse oximeter, and blood pressure cuff were placed to measure body temperature, blood oxygen level, blood pressure, and heart rate. Respiratory rate was monitored manually. 500 µL of blood was drawn for a baseline measurement of complete blood count (CBC), biodistribution, blood chemistry, coagulation panel, and cytokine levels. A syringe pump was used to administer the treatment intravenously via jugular at predetermined concentration at a rate of 1 mL per minute. Vitals were monitored every minute for the first 5 minutes, every 3 minutes for the next 15 minutes, and every 5 minutes for the remaining 40 minutes. Blood was also drawn at 2, 6, and 24 hours after administration of the various treatment groups. AT 24 hours, animals were euthanized with 100 mg/kg pentobarbital and organs (heart, spleen, liver, lung, kidney) were harvested for subsequent histopathology, immunofluorescence, and biodistribution studies.

Results for safety studies for I.V.-administered SP: We have started our studies with Lyo-SP dosing first, since it is Lyo-SP that is envisioned to be translated into field-deployable product for hemorrhage control. Vehicle (buffer) and Liq-SP will be studied subsequently. **Table 1** summarizes the survival of the groups we have completed thus far using Lyo-SP. As of the current groups, we have observed survival of all animals, so thus we conclude that we have not yet reached the maximum tolerated dose (MTD) of Lyo-SP, so we will continue with increasing dosage.

Table 1

Dosage	Survival
0 mg/kg (vehicle)	5/5 survived
0.1 mg/kg Lyo-SP	5/5 survived
1 mg/kg Lyo-SP	5/5 survived
10 mg/kg Lyo-SP	5/5 survived

Table 2 below shows representative data of complete blood counts (CBC) for the treatment groups drawn at the final time point. Missing data is due to some blood samples clotting prior to being analyzed. The abbreviations used below are the following: white blood cells (WBC), neutrophils (NE), lymphocytes (LY), monocytes (MO), eosinophils (EO), basophils (BA), red blood cells (RBC) hemoglobin (HB), hematocrit (HCT), mean cell volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin (MCHC), red blood cell distribution width (RDW), platelets (PLT), mean platelet volume (MPV).

Table 2:

IV Treatment	WBC (K/uL)	NE (K/uL)	LY (K/uL)	MO (K/uL)	EO (K/uL)	BA (K/uL)	NE %	LY %	MO %	EO %	BA %	RBC (M/uL)	HB (g/dL)	HCT %	MCV (fL)	MCH (Pg)	MCHC (g/dL)	RDW %	PLT (K/uL)	MPV (fL)
Vehicle (DPBS)	9.76	1.19	7.94	0.38	0.15	0.10	12.18	81.36	3.89	1.57	1.00	6.55	12.10	39.90	60.90	18.50	30.30	17.20	562	6.80
	16.96	2.68	13.08	0.85	0.23	0.13	15.81	77.11	4.99	1.33	0.77	7.95	14.60	49.80	62.60	18.40	29.30	16.70	925	6.80
0.1 mg/kg SP in DPBS	3.30	0.30	2.85	0.14	0.01	0.00	9.09	86.37	4.31	0.23	0.00	7.55	14.50	50.40	66.70	19.20	28.80	18.50	671	7.00
	18.44	2.68	14.59	0.73	0.31	0.13	14.56	79.14	3.95	1.67	0.68	8.82	17.90	61.70	69.90	20.30	29.00	16.90	455	6.70
	16.24	1.70	13.45	1.04	0.06	0.00	10.45	82.79	6.41	0.37	0.00	9.50	18.10	61.60	64.80	19.10	29.40	18.00	597	6.60
	14.70	1.89	11.83	0.62	0.22	0.14	12.83	80.50	4.25	1.50	0.92	8.58	17.70	56.90	66.30	20.60	31.10	17.80	288	7.50
1 mg/kg SP in DPBS	9.06	1.58	6.58	0.52	0.26	0.11	17.45	72.64	5.75	2.92	1.24	7.65	14.20	46.20	60.40	18.60	30.70	18.40	1179	6.80
	8.20	0.88	6.80	0.43	0.06	0.03	10.72	82.97	5.25	0.72	0.34	5.35	9.10	32.40	60.50	17.00	28.10	15.40	260	6.30
10 mg/kg SynthoPlate in DPBS	9.92	1.50	7.38	0.66	0.29	0.08	15.14	74.40	6.65	2.96	0.85	6.91	11.70	41.50	60.00	16.90	28.20	18.10	253	7.20
	14.02	2.17	10.58	0.64	0.41	0.21	15.47	75.48	4.59	2.95	1.51	7.42	13.10	43.50	58.60	17.70	30.10	18.60	1304	6.70
	8.88	1.38	6.72	0.44	0.26	0.07	15.53	75.72	4.99	2.94	0.82	6.76	11.90	38.90	57.50	17.60	30.60	16.70	206	6.30
	10.58	1.06	8.89	0.52	0.08	0.02	10.00	84.07	4.92	0.80	0.21	6.92	11.70	42.10	60.80	16.90	27.80	15.80	355	6.40

Figure 10 below shows some representative early time points and the final measurement of vital signs in the 10 mg/kg group (highest dose tested so far). The data suggests that even at this dosage level, the vitals have not significantly changed with regards to respiratory rate (RR) and SpO_2, but the heart rate does increase after 1 minute. However, as we have observed no other effects in the rats with regards to distress or survival, we will continue to observe these vitals for correlation to other outcomes as we increase in dosage. Additional data that will be collected at the conclusion of all studies, such as biodistribution and cytokine levels, may also shed more light on physiological changes in the animals. These observations, analyses and findings will be reported in the next phase.

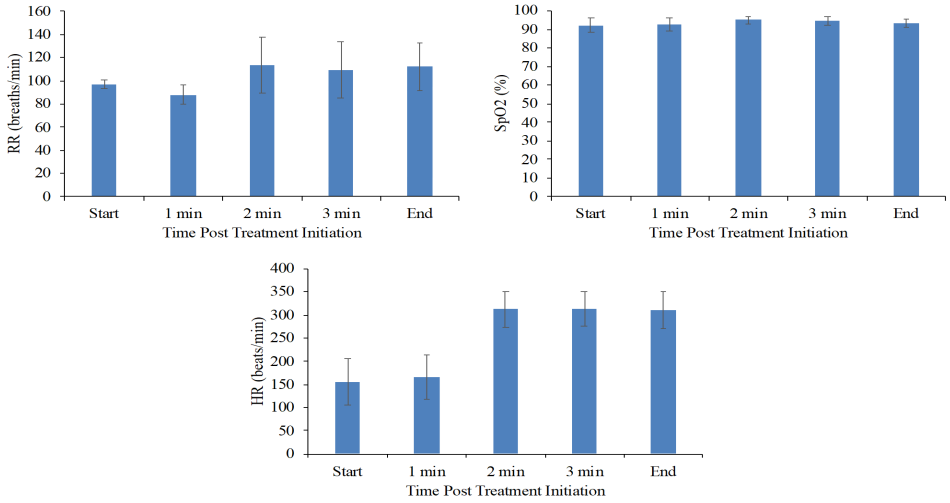

Figure 10. Representative vitals upon I.V.-administration of 10 mg/Kg Lyo-SP in rats.

Upon completion of the safety, biodistribution and MTD studies, the following 'efficacy evaluation' major tasks will be conducted during the 12-month NCE period:

Major Task 2: Evaluation of hemorrhage control efficacy and 3-day survival improvement in rats subjected to intraperitoneal hemorrhage by liver resection injury and intravenously dosed with saline suspension of SP or Lyo-SP at 25% and 50% of MTD at 5 min or 15 min post-injury.

Major Task 3: Feasibility evaluation of hemorrhage control efficacy and 24-hr survival improvem to intraperitoneal hemorrhage by liver resection injury and *intraosseously dosed* with Lyo-SP suspension at 25% and 50% of MTD at 5 min post-injury.

Specific Aim 3: Evaluation of systemic safety, method and time of delivery, hemostatic efficacy and survival outcome for reconstituted Lyo-SP compared to Liq- SP, **in rabbit intraperitoneal polytrauma hemorrhage model.**

Note: In the original SOW, the **Aim 3** studies were proposed to be conducted in a pig model of intraperitoneal hemorrhage. However, due to a variety of specific physiological issues that are present in cloven-hoofed animals (e.g. pig, sheep) that make them uniquely sensitive to nanoparticle systems in cardiopulmonary circulation, a model change was requested from pigs to rabbits. This model change was also approved along with the 12-month NCE and the rabbit model IACUC has been approved at UPitt. These IACUC documents along with ACURO Appendix form have been submitted to ACURO for further approval.

Describe the Regulatory Protocol and Activity Status (if applicable).
Describe the Protocol and Activity Status for sections a-c, as applicable, using the format described for each section. If there is nothing significant to report during this reporting period, state "Nothing to Report."

(a) ***Human Use Regulatory Protocols***

TOTAL PROTOCOLS: *State the total number of human use protocols required to complete this project (e.g., 5 human subject research protocols will be required to complete the Statement of Work."). If not applicable, write "No human subjects research will be performed to complete the Statement of Work."*

PROTOCOL(S): *List the identifier and title for all human use protocols needed to complete the project. Include information about the approved target number for clinical significance, type of submission, type of approval with associated dates, and performance status.*

The following format shall be used:

Protocol (1 of 2 total):

Protocol [HRPO Assigned Number]: **E01542.1a**

Title: **Analysis of Nanoparticle Interactions with Blood Cells**

Target required for clinical significance: N/A as this is for in vitro analysis only, with blood from healthy blood donors for the purpose of isolating plasma and platelets for BioFlux studies in vitro

Target approved for clinical significance:

Submitted to and Approved by: Case Western IRB approved on April 16, 2020. HRPO approval memorandum received on June 30, 2020.

Provide bullet point list of protocol development, submission, amendments, and approvals (include IRB in addition to HRPO).

Status: Approved

Report (i) progress on subject recruitment, screening, enrollment, completion, and numbers of each compared to original planned target(s), e.g., number of subjects enrolled versus total number proposed; (ii) amendments submitted to the IRB and USAMRMC HRPO for review; and (iii) any adverse event/unanticipated problems involving risks to subjects or others and actions or plans for mitigation.

Nothing to report as all platelet studies were done with de-identified blood sample obtained from the hematology core facility at Case Western, since recruiting blood donor in the midst of the continued COVID-19 pandemic to obtain blood samples as per the IRB-approved and HRPO-approved protocol was not possible. Therefore, as an alternative we opted to obtain de-identified blood samples from the hematology core facility of Case Western (https://case.edu/cancer/research/shared-resources/hematopoietic-biorepository-and-cellular-therapy) which does not require usage of our own IRB. This has allowed the preliminary BioFlux studies with PRP isolated from the blood.

Protocol (2 of 2 total):

Protocol [HRPO Assigned Number]: **E01542.2a**

Title: **Evaluation of Platelet Mitochondrial Function After Trauma in Human Adults**

Target required for clinical significance:

Target approved for clinical significance:

Submitted to and Approved by: HRPO approval memorandum received on July 28, 2020.

Provide bullet point list of protocol development, submission, amendments, and approvals (include IRB in addition to HRPO).

Status: Approved

Report (i) progress on subject recruitment, screening, enrollment, completion, and numbers of each compared to original planned target(s), e.g., number of subjects enrolled versus total number proposed; (ii) amendments submitted to the IRB and USAMRMC HRPO for review; and (iii) any adverse event/unanticipated problems involving risks to subjects or others and actions or plans for mitigation.

Nothing to report as these studies have not yet been started at UPitt.

(b) Use of Human Cadavers for Research Development Test & Evaluation (RDT&E), Education or Training

"Cadaver" is defined as a deceased person or portion thereof, and is synonymous with the terms "human cadaver" and "post-mortem human subject" or "PMHS." The term includes organs, tissues, eyes, bones, arteries or other specimens obtained from an individual upon or after death. The term "cadaver" does not include portions of an individual person, such as organs, tissue or blood, that were removed while the individual was alive (for example, if a living person donated tissue for use in future research protocols, that tissue is not considered a "cadaver" under this policy, regardless of whether the donor is living or deceased at the time of tissue use).

TOTAL ACTIVITIES*: State the total number of RDT&E, education or training activities that will involve cadavers. If not applicable, write "No RDT&E, education or training activities involving human cadavers will be performed to complete the Statement of Work (SOW)."*

ACTIVITIES: *Provide the following information in a bulleted list for all RDT&E, education or training activities involving human cadavers conducted or supported during the quarter:*

- *Title of the RDT&E, education or training activity*
- *SOW task/aim associated with the activity*
- *Date the activity was conducted*
- *Identification of the organization's responsible individual (e.g., PI or individual primarily responsible for the activity's conduct)*
- *Brief description of the use(s) of cadavers in the activity and the total number of cadavers used during the reporting period*
- *Brief description of the Department of Army organization's involvement in the activity*
- *Status of document submission and approvals*
- *Problems encountered in the procurement, inventory, use, storage, transfer, transportation and disposition of cadavers used for RDT&E, education or training. Examples of problems include but are not limited to: loss of confidentiality of cadaveric donors, breach of security, significant deviation from the approved protocol, failure to comply with state laws and/or institutional policies and public relations issues.*

(c) Animal Use Regulatory Protocols

TOTAL PROTOCOL(S):

State the total number of animal use protocols required to complete this project (e.g., 2 animal use research protocols will be required to complete the Statement of Work.). If not applicable, write "No animal use research will be performed to complete the Statement of Work."

2 protocols, one at CWRU and one at UPitt.

PROTOCOL(S):

List the identifier and title for all animal use protocols needed to complete the project. Include information about the approved target number for statistical significance, type of submission, type of approval with associated dates, and performance status.

The following format shall be used:

Protocol (1 of total 2):

Protocol [ACURO Assigned Number]: **ACURO protocol PR191632.e001**
Case Western IACUC Protocol # 2017-0102
Title: **Augmenting Hemostasis and Survival in Trauma by Targeted Drug Delivery**
Target required for statistical significance:
Target approved for statistical significance:
IACUC submitted to ACURO.

Status:

Approved. The rat studies are ongoing and will continue in the subsequent quarters.

Protocol (2 of total 2):

Protocol [ACURO Assigned Number]: **ACURO protocol PR191632.e002**

University of Pittsburgh IACUC Protocol Number: #22050418

Title: **Evaluation of intraosseous administration of lyophilized synthetic platelets in a rat liver hemorrhage model**

Status:

Approved. The rat studies will begin in September 2023 and be completed in 3 months.

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.

The project enabled training and professional development opportunity for multiple scientists and researchers at Haima Therapeutics (Michael Bruckman, Christa Pawlowski, Andrew Ditto, Shrijal Desai, Sana Sayed, Ujjal Sekhon, Baylee Traylor), at Sen Gupta laboratory at Case Western (Anirban Sen Gupta, Nathan Rohner, Norman Luc, Dante Disharoon), and at Neal laboratory at University of Pittsburgh (Matthew Neal, Roberto Mota Alvarez, Susan Shea, Emily Mihalko, Rassam Rassam). Under the supervision of Bruckman (Haima) Sen Gupta (Case Western), and Neal (UPitt) the researchers were able to undergo training in operational aspects of nanoparticle manufacture, lyophilization, physical property (size, charge) characterization and bioactive property (adhesion, aggregation) characterization, microfluidics and rat model studies. They executed all studies, acquired data, analyzed data and prepared scientific documents for progress report, MHSRS and CTTACC posters, and annual report.

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

Podium Presentations:

1. HERETIC Symposium at UPitt: https://www.ttmrc.pitt.edu/enrichment/heretic
Current Status of Platelet Mimetics in Bleeding management. Presented by Dr. Anirban Sen Gupta on October 12, 2022.

2. CTTACC Symposium in Scottsdale: https://cttacc.org
Synthetic Platelets for Use in Trauma and Hemorrhage. Presented by Dr. Anirban Sen Gupta on May 9, 2023.

Poster Presentations:

1. Cell Biology of Platelets and Megakaryocyte Gordon Conference 2023: https://www.grc.org/cell-biology-of-megakaryocytes-and-platelets-grs-conference/2023/

Lyophilized Synthetic Platelet (Lyo-SP) Nanotechnology as a Portable Storage-stable Rapidly Reconstitutable Intravenous Hemostat for Hemorrhage Control

[1]Shrijal S. Desai, [1]Ujjal Didar Singh Sekhon PhD, [2]Amudan J. Srinivasan MD, [1]Sana Syed PhD, [3]Dante Disharoon PhD, [1]Emily Gahagan, [1]Brittany Tatum, [1]Baylee Traylor, [1]Alexander Dornback, [2]Zachary A. Secunda MD, [2]Roberto I. Mota-Alvidrez MD, [3]Norman F. Luc, [1]Andrew Ditto PhD,[1]Christa L. Pawlowski PhD, [2]Matthew D. Neal MD, [1]Michael Bruckman PhD, [1,3]Anirban Sen Gupta PhD *
[1]Haima Therapeutics LLC, Cleveland, OH
[2]Trauma and Transfusion Medicine Research Center, Univ. of Pittsburgh, Pittsburgh, PA
[3]Dept. of Biomedical Engineering, Case Western Reserve University, Cleveland, OH

2. MHSRS 2023: https://mhsrs.health.mil/SitePages/Home.aspx

Lyophilizable Field-deployable Intravenous Platelet Surrogate Nanotechnology Evaluation in Rabbit Models of Bleeding

[1]Ujjal Didar Singh Sekhon PhD, [1]Sana Syed PhD, [2]Amudan J. Srinivasan MD, [2]Zachary A. Secunda, [1]Shrijal Desai, [3]Dante Disharoon PhD, [2]Roberto I. Mota Alvidrez MD, [1]Baylee Traylor, [1]Emily Gahagan, [3]Norman F. Luc, [4]Jane Arthur PhD, [4]Wilfried Krege, [5]Frauke May PhD, [5]Markus Brechmann PhD, [2]Joshua B. Brown MD FACS, [2]Philip C. Spinella MD, [2]Susan M. Shea PhD, [1]Christa L. Pawlowski PhD, [2]Matthew D. Neal MD, [1]Michael Bruckman PhD, [1,3]Anirban Sen Gupta PhD *
[1]Haima Therapeutics LLC, Cleveland, OH
[2]Trauma and Transfusion Medicine Research Center, Univ. of Pittsburgh, Pittsburgh, PA
[3]Dept. of Biomedical Engineering, Case Western Reserve University, Cleveland, OH
[4]CSL Innovation, Melbourne, Australia
[5]CSL Behring Innovation GmbH, Marburg, Germany

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

During the next reporting period (Year 4 with 12-month NCE) the following major aspects are anticipated to be executed and completed:

- **Specific Aim 1, Major Task 3:** In vitro evaluation of the effect of Lyo-SP versus Liq-SP in trauma patient blood samples
- **Specific Aim 2, All Tasks:** Safety and efficacy studies of Liq-SP versus Lyo-SP in intravenous dosing in rats; Safety and efficacy studies of Liq-SP versus Lyo-SP in intraosseous dosing in rats.
- **Revised Specific Aim 3, All Tasks:** Safety and Efficacy studies in rabbit trauma model
- Submission of **3 research manuscripts**, to peer-reviewed journals.

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).

The cumulative main impact of Year 1, 2 and 3 studies was the establishment of a validated process for effectively lyophilizing a synthetic platelet surrogate (SynthoPlate, SP to Lyophilized SynthoPlate, Lyo-SP) with reproducible quality and characteristics, and demonstrating conservation of hemostatic bioactivity of Lyo-SP upon aqueous reconstitution. These conserved functional aspects were demonstrated via multiple complementary in vitro assays. It was also demonstrated that Lyo-SP can be preserved for at least 12 months, and stays stable at a broad range of temperature between -20^{o}C and + 50^{o}C. These findings suggest that If successfully executed and translated, the Lyo-SP product can provide significant logistical and operational benefit as a field-deployable, easily portable, on-demand aqueous reconstitutable intravenous hemostatic agent for hemorrhage control in battlefield RDCR settings, and our continued studies under the current SOW will allow us to further advance this technology towards this milestone. An additional impact is evident from the fact that Lyo-SP has now been selected as a potential hemostatic component to be integrated with a lyophilized RBC-mimicking technology (e.g. Erythromer from Kalocyte) and a dry plasma technology (e.g. freeze-dried plasma from Teleflex or spray-dried plasma from Velico) to create a lyophilized whole blood analogue, supported by the DARPA FSHARP endeavor (https://www.darpa.mil/news-events/2023-01-31).

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.

The findings may influence the methodology and process development of other therapeutic products based on lipidic nanoparticles (e.g. chemotherapies, vaccine LNPs etc.) to achieve lyophilized versions of these products that are easily portable in pre-hospital settings without the need of special containers and cold chain, to help management of various pathological conditions. The findings also provide methodological insight into evaluation of manufacturing process, safety and efficacy of lyophilized nanoparticle systems in general.

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.*

The lyophilization method development for Lyo-SP is anticipated to result in new Intellectual Property related to lyophilization formulations and process conditions. Haima Therapeutics, the manufacturing entity for Lyo-SP, is developing and submitting new potential IP.

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.*

- Several reports have been recently published in magazines and news channels regarding the global need for blood surrogate technologies for managing a variety of bleeding complications and in such reports SynthoPlate has been highlighted as a potential example:

  https://www.vox.com/future-perfect/23683611/lab-grown-blood-trial-shortages-darpa

  https://thedaily.case.edu/quest-for-blood-surrogate-gets-46-million-boost-case-western-reserve-plays-key-research-role/

  https://www.aabb.org/news-resources/news/article/2023/03/20/aabb-news-biosynthetic-components-come-together-in-search-for-whole-blood-substitutes

  https://fox8.com/news/health/cleveland-plays-a-big-role-in-a-life-saving-world-first-medical-innovation-artificial-blood/

- Training of female and URM researchers to enhance the equity, inclusivity and diversity of research workforce in areas important to DOD mission and tasks.

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:*

The predominant problem in executing the project tasks in their early phase was the complete shutdown of experimental operations during whole of 2020 and initial few months of 2021 due to COVID-19 related restrictions. Operations were resumed in early 2021 and significant efforts were made to accelerate the pace of the task execution to 'catch up' to the proposed SOW as much as possible. A 12-month NCE request was submitted in July 2023 and was approved.

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.

The delays that were imparted due to COVID-19 related restrictions during the first 12 months of the Project beginning (July-December 2020, early Spring 2021) has resulted in a lag period regarding execution of Tasks and Sub-tasks as described in SOW, and this lag is anticipated to carry over as the project operations progress. A 12-month No Cost Extension (NCE) was requested by the PI (Sen Gupta) to complete all remaining tasks of the project and this was approved with the anticipated end date now to be July 2024 instead of July 2023. A model change from pigs to rabbit for Specific Aim 3 was also approved within this NCE period.

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.

N/A

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

Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

N/A

Significant changes in use or care of vertebrate animals

For Specific Aim 3 of the proposed SOW, a model change was requested from pig trauma hemorrhage model to rabbit trauma hemorrhage model. This is due to the presence of a variety of specific physiological issues in cloven-hoofed animals (e.g. pig, sheep) that make them uniquely sensitive and unsuitable to test intravenously dosed nanoparticle systems in cardiopulmonary circulation. This model change was also approved along with the 12-month NCE and the rabbit model IACUC has been approved at UPitt. These IACUC documents along with ACURO Appendix form have been submitted to ACURO for further approval.

Significant changes in use of biohazards and/or select agents

N/A

6. Products: List any products resulting from the project during the reporting period. If there are no products to report for the current quarter, state "Nothing to report."

Examples of products include:

Podium Presentations:

1. HERETIC Symposium at UPitt: https://www.ttmrc.pitt.edu/enrichment/heretic
Current Status of Platelet Mimetics in Bleeding management. Presented by Dr. Anirban Sen Gupta on October 12, 2022.

2. CTTACC Symposium in Scottsdale: https://cttacc.org
Synthetic Platelets for Use in Trauma and Hemorrhage. Presented by Dr. Anirban Sen Gupta on May 9, 2023.

Research poster for Platelet and Megakaryocyte Gordon Conference 2023:
Lyophilized Synthetic Platelet (Lyo-SP) Nanotechnology as a Portable Storage-stable Rapidly Reconstitutable Intravenous Hemostat for Hemorrhage Control

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[1]Haima Therapeutics LLC, Cleveland, OH
[2]Trauma and Transfusion Medicine Research Center, Univ. of Pittsburgh, Pittsburgh, PA
[3]Dept. of Biomedical Engineering, Case Western Reserve University, Cleveland, OH

Research poster for MHSRS 2023:
Lyophilizable Field-deployable Intravenous Platelet Surrogate Nanotechnology Evaluation in Rabbit Models of Bleeding

[1]Ujjal Didar Singh Sekhon PhD, [1]Sana Syed PhD, [2]Amudan J. Srinivasan MD, [2]Zachary A. Secunda, [1]Shrijal Desai, [3]Dante Disharoon PhD, [2]Roberto I. Mota Alvidrez MD, [1]Baylee Traylor, [1]Emily Gahagan, [3]Norman F. Luc, [4]Jane Arthur PhD, [4]Wilfried Krege, [5]Frauke May PhD, [5]Markus Brechmann PhD, [2]Joshua B. Brown MD FACS, [2]Philip C. Spinella MD, [2]Susan M. Shea PhD, [1]Christa L. Pawlowski PhD, [2]Matthew D. Neal MD, [1]Michael Bruckman PhD, [1,3]Anirban Sen Gupta PhD *

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[4]CSL Innovation, Melbourne, Australia
[5]CSL Behring Innovation GmbH, Marburg, Germany

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".

Anirban Sen Gupta, PhD
Project Role: PD/PI
Research Identifier: https://orcid.org/0000-0002-5773-0667
Nearest person month worked: 3
Contribution to Project: Dr. Sen Gupta provided overall direction and guidance, for experimental design, execution and analysis for studies proposed under the Specific Aims of the project. Dr. Sen Gupta also prepared and submitted quarterly progress report. In addition, Dr. Sen Gupta carried out communication and planning with collaborators at Haima and UPitt for subsequent phases of the project that will be carried out at current location.

Norman Luc
Project Role: Researcher at CWRU in Sen Gupta lab
Research Identifier:
Nearest person month worked: 12
Contribution to Project: Mr. Luc contributed to assisting in assisting with DSC and Litesizer analysis of SP and Lyo-SP.

Nathan Rohner, PhD
Project Role: Senior Research Associate at CWRU in Sen Gupta lab
Research Identifier:
Nearest person month worked: 8
Contribution to Project: Dr. Rohner oversaw procurement, installation and training of DSC and Litesizer in Sen Gupta lab, and assisted with SP and Lyo-SP analysis on these instruments.

Dante Disharoon, PhD
Project Role: Research Associate at CWRU in Sen Gupta lab
Research Identifier:
Nearest person month worked: 4
Contribution to Project: Dr. Disharoon oversaw BioFlux systems and experiments in Sen Gupta lab.

Name: **Michael Bruckman**
Project Role: PI (CEO at Haima)
Nearest person month worked: 3
Contribution to Project: Michael managed the project operations, updated the budget documents, and assisted in any protocol development and results evaluation.

Name: **Christa Pawlowski**
Project Role: Key Personnel (COO at Haima)
Nearest person month worked: 2
Contribution to Project: Christa assisted with managing the project operations and assisted in any protocol development and results evaluation. Dr. Pawlowski led the subaward and subcontract document preparation. Finally, she assisted in developing the document control system and template documents for Haima.

Name: **Ujjal Didar Singh Sekhon**
Project Role: Key Personnel (Senior Scientist at Haima)
Nearest person month worked: 3
Contribution to Project: Andrew assisted with project management with Michael and Christa. He led the Cryo-TEM experiments. In the past quarter, he also helped get the clean room installed. Andrew is also leading the lyophilization process method development studies.

Name: **Emily Gahagan**
Project Role: Other Personnel (Research Scientist at Haima)
Nearest person month worked: 5
Contribution to Project: Emily executed the SynthoPlate physicochemical and functional characterization experiments.

Name: **Shrijal Desai**
Project Role: Other Personnel (Research Scientist at Haima)
Nearest person month worked: 3
Contribution to Project: Shrijal executed any required SynthoPlate manufacturing and also assists in any physicochemical and functional characterization.

Name: **Baylee Traylor**
Project Role: Other Personnel (Research Scientist at Haima)
Nearest person month worked: 3
Contribution to Project: Baylee executed any required SynthoPlate manufacturing and also assists in any physicochemical and functional characterization.

Name: Matthew D. Neal, MD
Project Role: co-I
Research Identifier:
Nearest person month worked: 1
Contribution to Project: Dr. Neal leads the experimental design and analysis for trauma patient blood studies in vitro and will be responsible for intraosseous delivery studies in rats (Aim 2) and downstream other in vivo studies. He meets regularly with Dr. Sen Gupta and his team via phone and ZOOM for planning and execution of the proposed studies.

Name: Roberto Mota-Alvarez
Project Role: Research Scientist
Nearest person month worked: 1
Contribution to Project: Dr. Mota-Alvarez is involved in the proposed in vivo studies.

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.

The following new grants were awarded to the PI (Sen Gupta) during this reporting period but this has not altered his effort % contribution to PR191632.

1. DoD PRMRP IIRA PR201584 Sen Gupta (Co-I) 09/2021- 08/2024
Total direct:
TraumaChek™: A Field-deployable Dielectric Coagulometer for Comprehensive Hemostatic Assessment In Remote Damage Control Resuscitation (RDCR)
The overall goal of the proposal is to design and evaluate a multichannel dielectric coagulometry microsensor device for rapid point-of-injury assessment of trauma-induced coagulopathy.

Agency Contact: Dr. Robin Walker

2. DoD PRMRP IIRA PR211157 Sen Gupta (PI) 08/2022 – 07/2026 Total Direct:
SanguiStop: Intravenous Nanomedicine for Targeted Thrombin Delivery in Hemorrhage Control
The overall goal is to design and evaluate a platelet-inspired nanomedicine system that enables direct delivery of thrombin in a targeted fashion to bleeding injury site, to circumvent coagulation and platelet dysfunctions and rescue fibrin status for hemostatic efficacy in hemorrhage control.
Agency Contact: Dr. Darrell Ellsworth: _____

3. DARPA FSHARP Contract Sen Gupta (Subcontract Lead at CWRU) 03/2023 – 02/2027
Total Direct for CWRU subcontract: (Sen Gupta 0.33 Mo Academic; 1 Mo Summer) ***CONCERT: Consortium for Optimized Integration of Bioartificial Blood Components for Adaptive Resuscitation and Therapy***
Central Aim: The overall goal is to integrate and optimize lyophilizable artificial red cell, artificial platelet and freeze-dried plasma systems to create a field-deployable powder-form rapidly aqueous reconstitutable biosynthetic whole blood surrogate design and evaluate this design in rabbit and NHP models for hemostatic resuscitation in trauma-induced coagulopathy.
Agency contact: Dr. Jean-Paul Chretien

None of the above awards have any overlap with PR191632 but are rather complementary to the milestones of PR191632, with the broader mission of advancing field-deployable therapeutic and diagnostic technologies for point-of-injury trauma management in the battlefield.

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.

Lead organization: Case Western
Collaborating organizations: Haima Therapeutics, University of Pittsburgh

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://ebrap.org/eBRAP/public/index.htm*** *for each unique award.*

QUAD CHARTS: *If applicable, the Quad Chart (available on https://www.usamraa.army.mil/Pages/Resources.aspx) 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.*
10.