AWARD NUMBER: W81XWH-20-1-0628

TITLE: Field-Deployable Dried Platelet Surrogate Nanotechnology for Hemorrhage Control in RDCR

PRINCIPAL INVESTIGATOR: Anirban Sen Gupta

CONTRACTING ORGANIZATION: Case Western Reserve University

REPORT DATE: AUGUST 2022

TYPE OF REPORT: Annual Progress Report

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

DISTRIBUTION STATEMENT: Approved for public release; distribution is unlimited.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	**2. REPORT TYPE**	**3. DATES COVERED**
AUGUST 2022	Annual Progress Report	07/15/2021–07/14/2022

4. TITLE AND SUBTITLE

Field-Deployable Dried Platelet Surrogate Nanotechnology for Hemorrhage Control in RDCR

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-20-1-0628

5c. PROGRAM ELEMENT NUMBER

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

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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

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.

15. SUBJECT TERMS

NONE LISTED

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
Unclassified	Unclassified	Unclassified	Unclassified	**24**	**19b. TELEPHONE NUMBER** *(include area code)*

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

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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 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.

The overall focus of the project is to evaluate, optimize and establish a nanoparticle-based lyophilized synthetic platelet surrogate for hemorrhage control and hemostatic resuscitation in far forward battlefield setting in the framework of Remote Damage Control Resuscitation (RDCR). The 'synthetic platelet' nanotechnology in focus here is called SynthoPlate (SP) and the lyophilized form is termed Lyo-SP.

The evaluation is to be carried out under the following Specific Aims:
Aim 1: In vitro evaluation of platelet-relevant bioactivity for Lyo-SP compared to freshly made non-lyophilized SP, utilizing microfluidics, aggregometry, flow cytometry and ROTEM, utilizing human (healthy donor and trauma patient) plasma and whole blood.

Aim 2: Evaluation of systemic safety, hemostatic efficacy and survival outcome for reconstituted Lyo-SP compared to non-lyophilized SP, varying dosage and time-of-administration in rat liver hemorrhage model.

Aim 3: Evaluation of systemic safety, method and time of delivery, hemostatic efficacy and survival outcome for reconstituted Lyo-SP compared to non-lyophilized SP, in pig intraperitoneal hemorrhage model.

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.

1. Major Activities:

In the period of 07/2021 – 07/2022, the Major Tasks (and associated Subtasks) were conducted as follows:

- **For Sp Aim 1, Major Task 1,** all subtasks (except Lexamed sterilization characterization) were completed in Year 1, and results are compiled in Year 1 Progress Reports as well as Year 1 Annual Report. SP and Lyo-SP have now been shipped to LexaMed (Toledo, Ohio) for sterilization by E-Beam and shipment back to CWRU for characterization studies (awaiting return shipment).

- **For Sp Aim 1, Major Task 2 (all subtasks)** :
  i. Studies were continued with SP and Lyo-SP manufactured by Haima Therapeutics (as per process established in Year 1) and transferred back to Sen Gupta lab (CWRU) for characterization of the shape/size/morphology of the resultant nanoparticles as fresh-manufactured system as well as 'stored' system to establish the optimized lyophilization conditions and ideal lyoprotectant usage.
  ii. Studies were continued on DLS and Cryo-TEM of sterilized SP suspension vs aqueous reconstituted sterilized Lyo-SP for size, morphology and stability characterization and comparison
  iii. BioFlux Microfluidics studies were continued for in vitro performance analysis of SP and Lyo-SP systems. Additionally, aggregometry and ROTEM analyses were continued regarding studying the effect of SP and Lyo-SP on platelet aggregation in plasma (aggregometry assays) and clot viscoelastometry in whole blood (ROTEM assays).

- **For Sp Aim 1, Major Task 3, Subtasks 1 and 2**:

  iv. SP and Lyo-SP were manufactured as per Year 1-established process for shipment to UPitt/UPMC
  v. IRB/HRPO approval for in vitro SP/Lyo-SP studies with trauma patient blood at UPitt was obtained
  vi. Microfluidics, aggregometry and viscoelastometric studies with SP vs Lyo-SP in trauma patient blood was initiated.

- **For Sp Aim 2, Major Task 1, Subtasks 1 and 2**:
  vii. IACUC/ACURO approval for rat studies at Case Western (safety and efficacy in liver hemorrhage model, with *intravenously* administered Liq-SP and Lyo-SP) was obtained
  viii. IACUC approval for rat studies at UPitt (efficacy in liver hemorrhage model, with *intraosseously* administered Liq-SP and Lyo-SP) was obtained, and is submitted for ACURO review and approval
  ix. Studies initiated at Case Western in rats for safety and efficacy studies with I.V.-dosed SP

2. Specific Objectives:

- **For Sp Aim 1, Major Task 2,** the specific objectives achieved during Year 2 are:

  i. Reproducible manufacture of Liq-SP and Lyo-SP by Haima Therapeutics as per protocol established in Year 1
  ii. Characterization of Size/Shape/Morphology of the manufactured Liq-SP and Lyo-SP fresh, and then of Lyo-SP upon storage at various temperature conditions.

iii. Microfluidic (BioFlux), Aggregometry (LTA) and Viscoelastometry (ROTEM/TEG) characterization of hemostatic augmenting property of Liq-SP and Lyo-SP.

- **For Sp Aim 1, Major Task 3,** the specific objectives achieved during Year 2 are:

i. Manufacture of SP and Lyo-SP were manufactured as per Year 1-established process for shipment to UPitt/UPMC
ii. IRB/HRPO approval for in vitro SP/Lyo-SP studies with trauma patient blood at UPitt was obtained
iii. Microfluidics, aggregometry and viscoelastometric studies with SP vs Lyo-SP in trauma patient blood was initiated.

- **For Sp Aim 2, Major Task 1,** the specific objectives achieved during Year 2 are:

i. IACUC/ACURO approval for rat studies at Case Western (safety and efficacy in liver hemorrhage model, with *intravenously* administered Liq-SP and Lyo-SP) was obtained
ii. IACUC approval for rat studies at UPitt (efficacy in liver hemorrhage model, with *intraosseously* administered Liq-SP and Lyo-SP) was obtained, and is submitted for ACURO review and approval
iii. Studies initiated at Case Western in rats for safety and efficacy studies with I.V.-dosed SP

3. Significant Results:

- **For Sp Aim 1, Major Task 2,** the significant results achieved during Year 2 are:

i. Establishment of scalable and reproducible lyophilization protocol for manufacturing Lyo-SP:

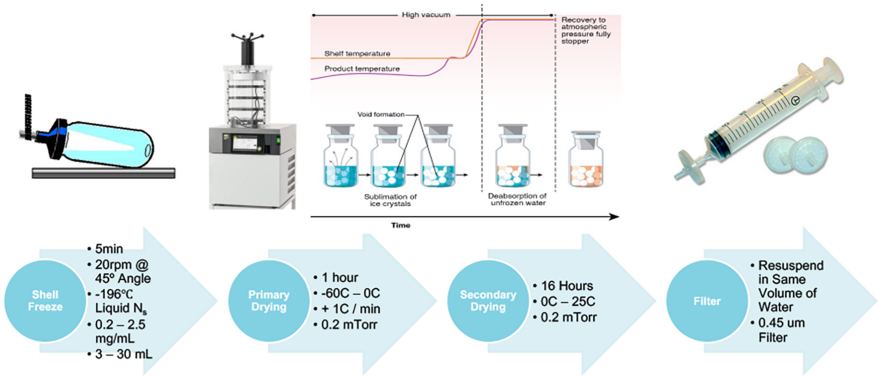


Figure 1. Lyophilization process used to manufacture Lyo-SP for subsequent characterization and in vitro analyses

Using this process, it was established that lyophilization of SP (Lyo-SP) preserves the size/shape/morphology of SP. Selected results are shown below (formulation reported in Progress Reports):

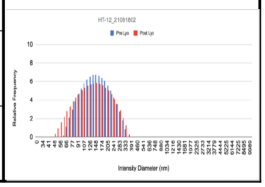
Stats	SP					Lyo-SP					Intensity Diameter Histogram
	Hydrodynamic Diameter (nm)	Intensity Diameter (nm)	Intensity D90 (nm)	PDI	Zeta Potential (mV)	Hydrodynamic Diameter (nm)	Intensity Diameter (nm)	Intensity D90 (nm)	PDI	Zeta Potential (mV)	
Mean (n=6)	237	177	277	0.29	-11	162	152	248	0.23	-9	
CV% (n=6)	13%	12%	22%	15%	20%	23%	19%	24%	14%	18%	
% Change Post-Lyo						-32%	-14%	-10%	-20%	-22%	

Table 1: Characterization of SP vs Lyo-SP formulations using DLS (size) and Zetasizer (charge)

ii. Using the lyophilization method of manufacturing Lyo-SP, it was also possible to study the 'variation' is Lyo-SP size/shape/morphology/stability stored over time (currently data is at 9 months). Representative results are shown below:

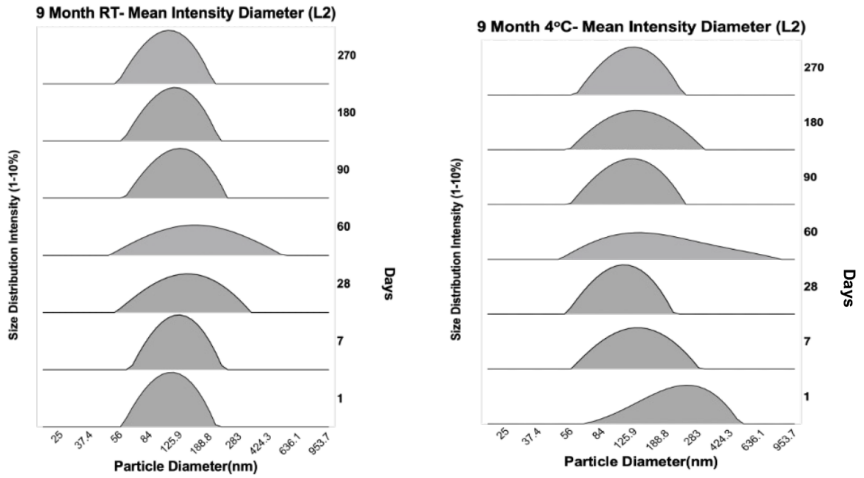


Figure 2. DLS data confirming that Lyo-SP stored at room temperature (RT) as well as refrigerator (4°C) maintains nanoparticle diameter for 270 days (9 months) and this is reflective of nanoparticle stability.

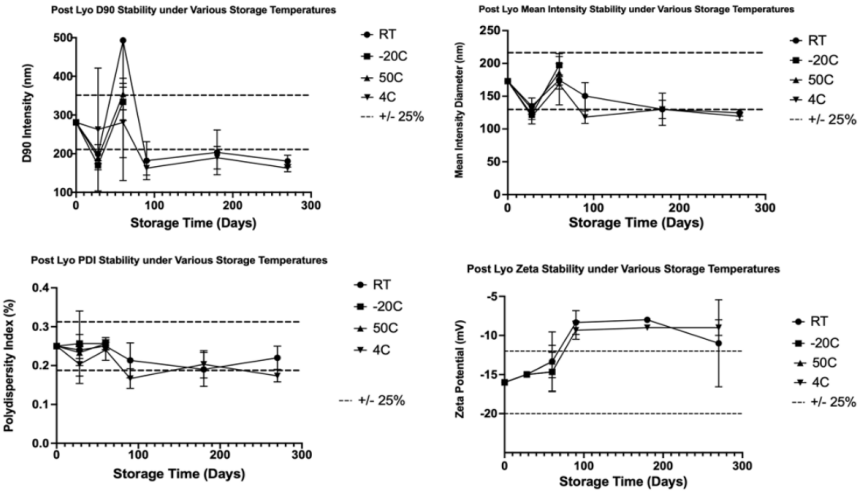


Figure 3. DLS data parameters corroborating the storage stability of Lyo-SP stored at various temperature conditions for 270 days (9 months).

iii. BioFlux microfluidic studies confirmed the capability of Lyo-SP to enhance the surface coverage of thrombocytopenic concentration of platelets on 'vWF + collagen'-coated microfluidic channels (simulating vWF and collagen exposure at a bleeding injury site), at the same level as Liq-SP. This demonstrates that lyophilization/reconstitution does not affect the platelet-mimetic hemostatic bioactivities of SP. BioFlux studies also indicated that the hemostatic augmentation of Liq-SP and Lyo-SP in thrombocytopenic (i.e. platelet-depleted) conditions is influenced by the particle: platelet ratio. The hemostatic augmentation at high shear flow condition was found to be predominantly at particle:platelet ratio of 1000: 1 or above (e.g. 5000: 1). Additionally, BioFlux studies indicated that the hemostatic augmentation effect of Liq-SP or Lyo-SP on thrombocytopenic platelet conditions are also influenced by shear, and the effect is predominant at high shear conditions but not as much in low shear conditions. Interestingly, traumatic injuries and associated acute hemorrhage are often high shear scenarios and therefore SP is expected to maintain its hemostatic effect in such conditions. Selected data are shown below, and further details are available in progress reports.

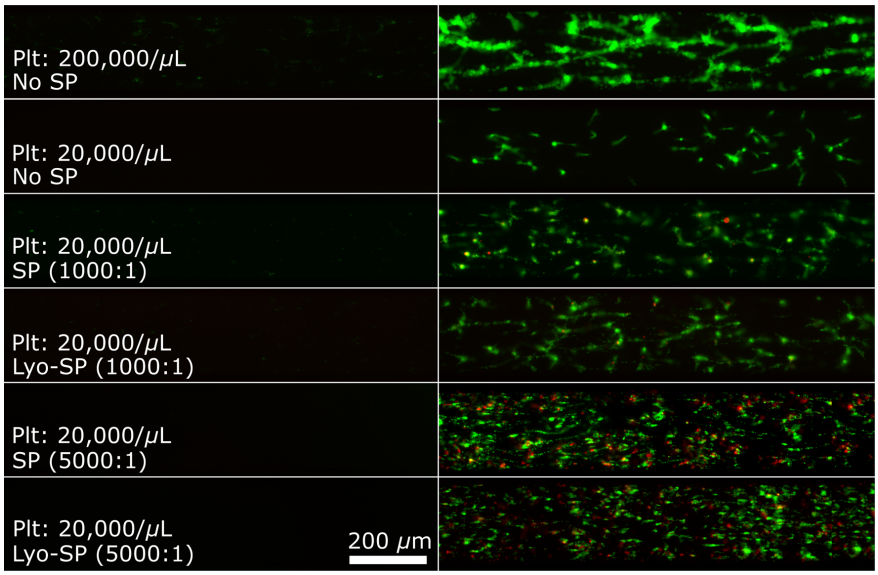


Figure 4. Representative BioFlux results indicating that Liq-SP and Lyo-SP can both improve the surface coverage of thrombocytopenic platelets on 'vWF + collagen' coated surfaces at comparable levels; Additionally, this hemostatic augmentation is increased at SP: Platelet ratios of 1000: 1 and above (e.g. 5000: 1).

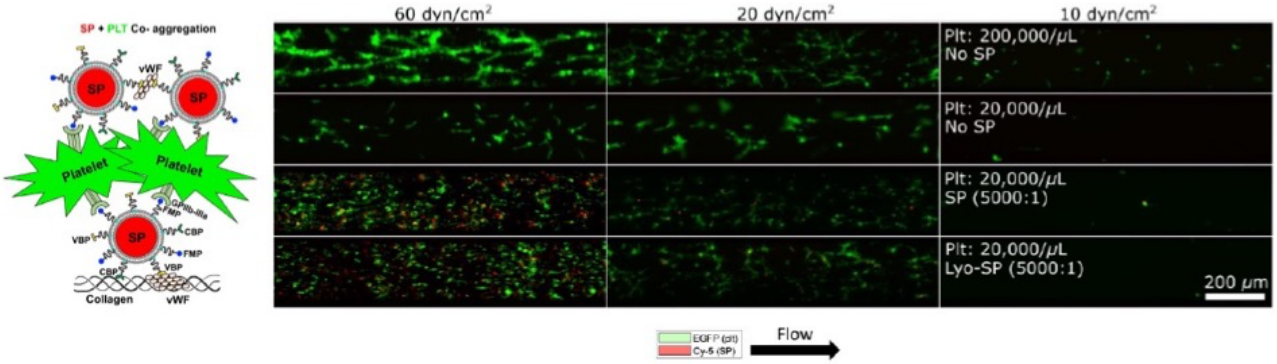


Figure 5. Representative BioFlux results indicate that Liq-SP and Lyo-SP can both improve the surface coverage of thrombocytopenic platelets on 'vWF + collagen' coated surfaces at comparable levels in high shear (60 dyn/cm^2) conditions, but this effect is substantially reduced at lower shear (e.g. 10 dyn/cm^2).

iv. Aggregometry studies confirmed that when platelet-rich plasma (PRP) is significantly depleted of platelets to form thrombocytopenic plasma, the aggregation % of platelets is severely reduced, and both Liq-SP and Lyo-SP can partly rescue the aggregation % of platelets in such thrombocytopenic condition:

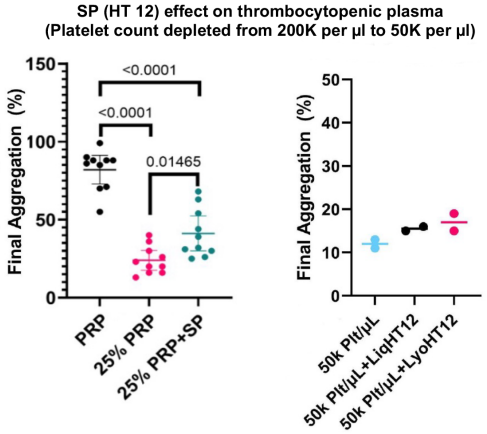


Figure 6. Representative aggregometry data indicate that SP can partly rescue/improve aggregation % of platelets in thrombocytopenic condition, and this capability is conserved at comparable levels between Liq-SP and Lyo-SP; HT 12 here is the label code of the SP manufactured by Haima Therapeutics.

The rescue effect in aggregometry assay is apparently not as high as indicated by microfluidic (BioFlux) assay. Our rationale is that this is because aggregometry is not a high shear flow condition like BioFlux, and thus the rescue is only modest.

v. Rotational Thromboelastometry (ROTEM) studies in NATEM mode further confirmed that lyophilization of SP (i.e. conversion of Liq-SP to Lyo-SP) conserves the hemostatic augmentation capability of SP in the context of rescuing clot viscoelastometric parameters and early clot kinetics, when PRP is depleted of platelets:

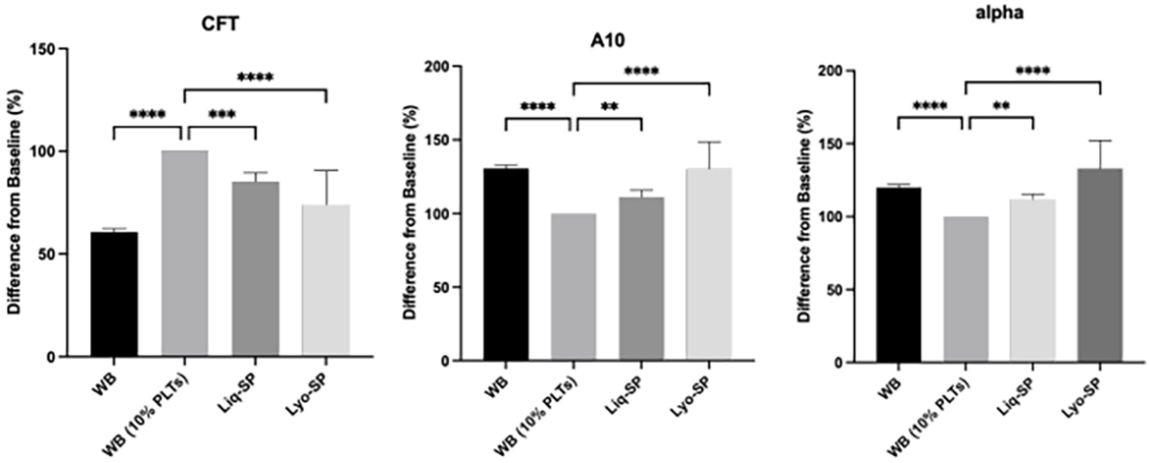


Figure 7. ROTEM NATEM studies in whole blood (WB) vs platelet-depleted WB (with only 10% platelets) showed that platelet depletion by 90% severely affects the early clot viscoelastometric kinetic properties (Clot Formation Time- CFT, A10 and Alpha angle) parameters; Treatment with Liq-SP or Lyo-SP comparably rescues these properties.

- **For Sp Aim 1, Major Task 3,** the significant results achieved during Year 2 are:

  i. IRB/HRPO approval for in vitro SP/Lyo-SP studies with trauma patient blood at UPitt was obtained and studies were initiated in a microfluidic system called the 'Bleed Chip' system:

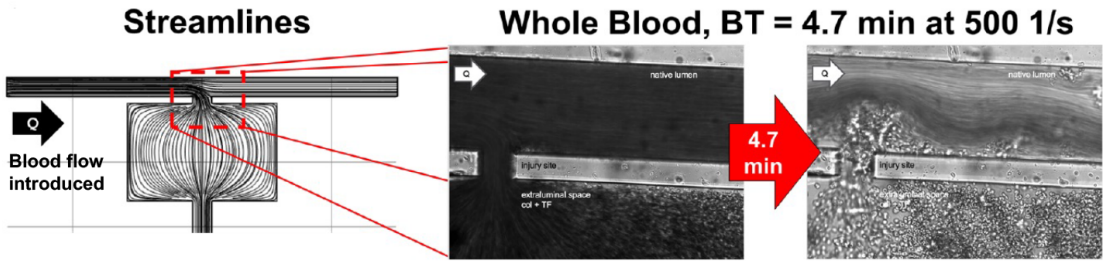


Figure 8. Schematic of Bleed Chip system and imaging of hemostatic clot formation

The microfluidic chamber (termed 'Bleed Chip') features a native lumen that is compromised, simulating injury, with an opening into an extraluminal space that is coated with extravascular proteins (collagen + TF) to simulate vessel wall injury. Fluid dynamic characterization and imaging of hemostatic clot formation in this system are shown above in **Figure 8**. As shown in **Figure 8**, streamlines visualized using computational fluid dynamics confirm flow pattern through the 'Bleed Chip' chamber. This system is already being used in the cold platelet CHIPS trial at UPitt, and therefore we rationalize that this system is appropriate for evaluating the hemostatic augmentation performance of Lyo-SP. The flow chamber (50 µm height throughout) is comprised of a lumen (150 µm width) with an outflow (50 µm width, "injury site") into a large extraluminal space (>5 times the lumen length and width).

The extraluminal space and injury site are both coated with collagen and tissue factor overnight at ambient temperature, then rinsed with PBS and the entire chamber blocked with PBS + 5% BSA for 30 minutes prior to use. Blood samples are first fileterd through a 40 μm filter to prevent emboli, then perfused via syringe pump through the bleeding chamber at initial wall shear rate of either 150 s^{-1} (low, venous) or 3500 s^{-1} (high, arterial). Calcium chloride ($CaCl_2$, #21115-250ML, Millipore-Sigma, St. Louis, MO, US) is perfused immediately upstream of the testbed at a 1:10 flowrate to achieve a final concentration of 10-15 mM in the chamber for recalcification. Occlusion is defined as the point at which clot formation sealed the injury site for at least three minutes, and the time from initial perfusion until occlusion is defined as the bleeding time (BT, s). If no seal is formed, the flow is stopped at 20 min, and the assay is given a BT of 1200 s. In **Figure 8**, blood flow is left to right. An example of hemostatic clot formation is shown where blood perfused through the chamber took 4.7 min to seal the 'injury site' (primary endpoint of bleeding time (BT)). Beyond measuring BT, the 'Bled Chip' can also be used to image/visualize the clot formation in real time and analyze clot composition. Here the clot formation at the 'injury site' is imaged in real time using a Zeiss Axio Observer 7 with transmitted light (TL) images taken at 0.62 frames per second. The number of pixels in the region of interest filled by the clot in each image is tabulated with a thresholding algorithm using MATLAB (version 2020a, The Math Works, Inc., Natick, MA, US). To visualize and quantify clot morphology and clot component contributions, fluorescently labeled antibodies are used towards clot components, and immunofluorescence microscopy imaging is used. Mean fluorescence intensity (MFI) from the images is calculated using MATLAB. Kinetic fluorescent curves are generated by normalizing the MFI of a given frame to the maximum MFI of the signal for the duration of the assay, to yield a % of maximum MFI value. The above two methods using the 'Bleed Chip' is expected to help analyze both 'kinetic' and 'compositional' effects of Lyo-SP in hemostatic clot formation in trauma patient blood.

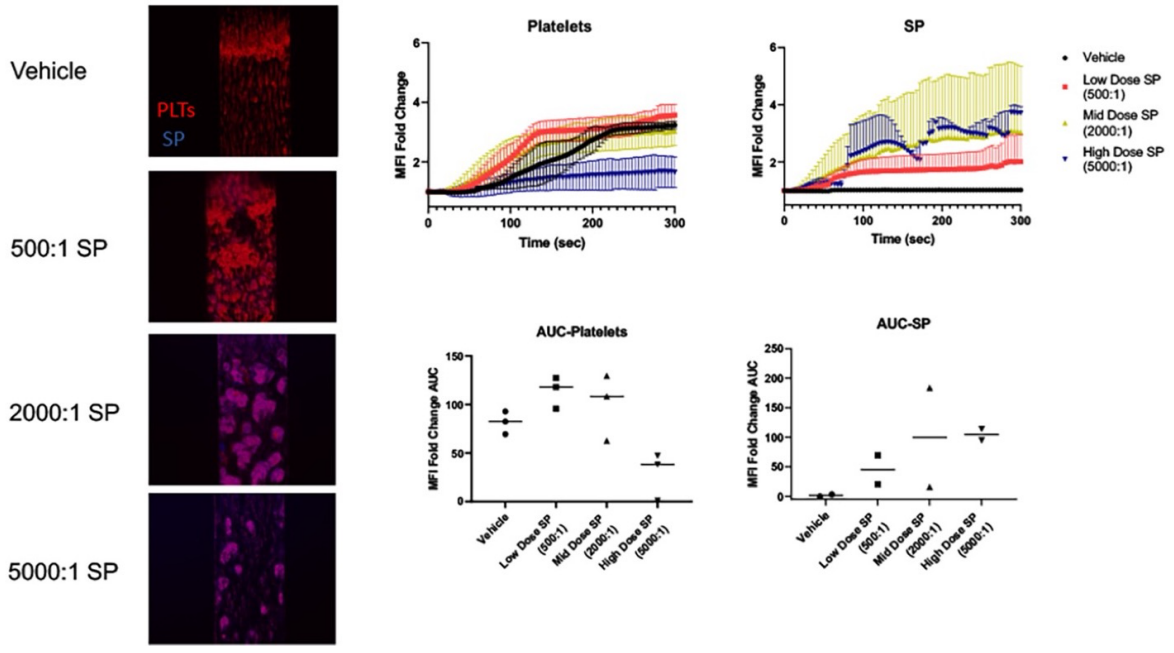


Figure 9. Preliminary baseline studies using microfluidics analyzing the effect of Lyo-SP on clot formation (composition and kinetics). SP seems to rescue clot formation in platelet-poor blood samples (reflective of platelet loss in trauma patient bleeding).

ii. Baseline TEG6S studies were also done by introducing Lyo-SP in whole blood. Here, whole blood (WB) was diluted (dWB) and Lyo-SP was added to assess whether Lyo-SP can improve hemostatic output of dWB. Preliminary study results are shown below:

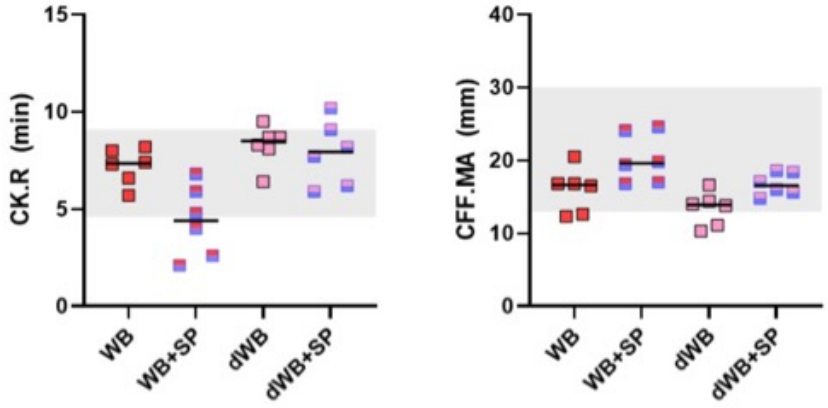


Figure 10. Preliminary TEG6S data to assess effect of Lyo-SP on dWB

As shown in **Figure 10**, inducing dilutional effect on WB (dWB) resulted in reduced Clotting Time (R) and Clot Amplitude (MA). Treatment of dWB with Lyo-SP seems to improve/rescue these parameters slightly. We will continue these studies at various Lyo-SP: Platelet ratios to test 'therapeutic dose range'.

- **For Sp Aim 2, Major Task 1,** the significant results achieved during Year 2 are:

  i. IACUC/ACURO approval for rat studies at Case Western (safety and efficacy in liver hemorrhage model, with *intravenously* administered Liq-SP and Lyo-SP) was obtained and rat safety studies were initiated (preliminary data shown below in **Figure 11**):

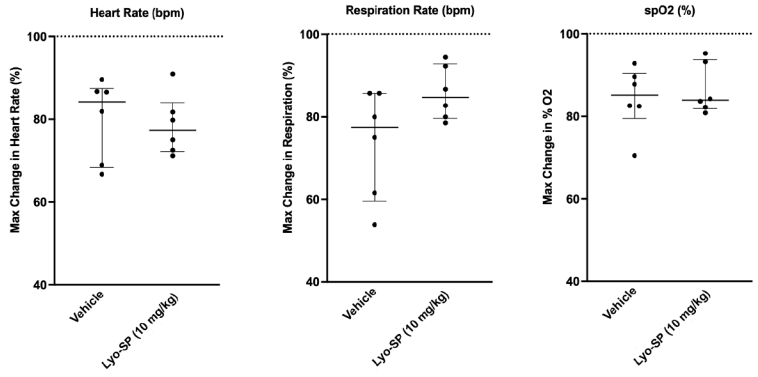


Figure 11. Representative vitals data upon IV dose of vehicle (saline only) vs Lyo-SP in Sprague-Dawley rats. At the current dose the Lyo-SP does not seem to render negative effect on rat vitals.

  ii. IACUC approval for rat studies at UPitt (efficacy in liver hemorrhage model, with *intraosseously* administered Liq-SP and Lyo-SP) was obtained, and is being submitted for ACURO review and approval. We anticipate approval and initiation of studies by October 2022.

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), at Sen Gupta laboratory at Case Western (Anirban Sen Gupta, Nathan Rohner, Norman Luc), and at Neal laboratory at University of Pittsburgh (Matthew Neal, Roberto Mota Alvarez, Susan Shea, Emily Mihalko). 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 this 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.

An abstract was submitted to MHSRS 2022 which has been accepted for podium presentation:

Lyophilized Synthetic Platelet (Lyo-SynthoPlate™) Technology for Far Forward Hemorrhage Control

Authors: [1]Shrijal Desai, [1]Andrew Ditto PhD, [1]Sana Syed PhD, [1]Ujjal DS Sekhon PhD, [1]Alex Dornback, [1]Baylee Traylor, [1]Emily Gahagan, [1]Nicole Rizkala, [2]Nathan Rohner PhD, [2]Dante Disharoon PhD, [2]Anirban Sen Gupta PhD*, [1]Christa Pawlowski PhD*, [1]Michael Bruckman PhD*

[1]Haima Therapeutics LLC, Cleveland, OH
[2]Dept of Biomedical Engineering, Case Western Reserve University, Cleveland, OH

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

During the next reporting period the three following major aspects are anticipated to be executed:
- In vitro evaluation of the effect of Lyo-SP versus Liq-SP in trauma patient blood samples
- 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
- Submission of at least 1, potentially 2 manuscripts, on 'Year 1 + Year 2' and Year 3 studies.

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 main Impact of Year 1 studies was the establishment of the potential of effectively lyophilizing a synthetic platelet surrogate (SynthoPlate, SP to Lyophilized SynthoPlate, Lyo-SP) with reproducible quality and characteristics, demonstrating conservation of hemostatic bioactivity. The main impact of Year 2 studies is the demonstration via multiple complementary in vitro assays that lyophilization of SP to Lyo-SP conserves its hemostatic ability while allowing storage for ~ 9 months at various temperature conditions. If successfully executed and translated, the Lyo-SP product can provide significant benefit as a field-deployable, easily portable, on-demand aqueous reconstitutable intravenous hemostatic agent for hemorrhage control in battlefield RDCR settings.

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 rationale to combine Lyo-SP with other feeze-dried hemostatic products (e.g. Freeze-dried plasma) and lyophilizable RBC surrogates to potentially create combinatorial resusuctative systems akin to whole blood.

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 in the process of 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.*

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:*

Changes in approach and reasons for change
Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

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. Although significant success was achieved to this end, there is still an existent lag regarding several sub-tasks that has persisted through 2022 due to reagent supply chain issues, university administrative employee turnover. A one year NCE request is anticipated to complete the tasks.

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. With this anticipation, the PI (Sen Gupta) is planning to request a 12-month No Cost Extension (NCE) to complete all the proposed tasks of the project (anticipated end date to be July 2024 instead of July 2023).

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.

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

Significant changes in use of biohazards and/or select agents

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."*

Nothing to Report.

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

An abstract was submitted to MHSRS 2022 which has been accepted for podium presentation:

Lyophilized Synthetic Platelet (Lyo-SynthoPlate™) Technology for Far Forward Hemorrhage Control

Authors: [1]Shrijal Desai, [1]Andrew Ditto PhD, [1]Sana Syed PhD, [1]Ujjal DS Sekhon PhD, [1]Alex Dornback, [1]Baylee Traylor, [1]Emily Gahagan, [1]Nicole Rizkala, [2]Nathan Rohner PhD, [2]Dante Disharoon PhD, [2]Anirban Sen Gupta PhD*, [1]Christa Pawlowski PhD*, [1]Michael Bruckman PhD*

[1]Haima Therapeutics LLC, Cleveland, OH
[2]Dept of Biomedical Engineering, Case Western Reserve University, Cleveland, OH

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).*

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).

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.*

- **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.

- **Technologies or techniques**
  Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

  - Optimization of 'vWF + Collagen'-coating technique in BioFlux microfluidic channels to allow consistency of platelet adhesion and SP effect analysis in PRP vs thrombocytopenic conditions at various shear flow conditions.
  - Optimization of the 'Bleed Chip' microfluidic system for analysis of SP effect on trauma patient blood

- **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.

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 in the process of developing and submitting new potential IP.

- **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.*

6. 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*

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: **Andrew Ditto**
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 II 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 I 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: 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."

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.

A PRMRP IIRA proposed project submitted by Dr. Sen Gupta in summer of 2021 was recommended for funding, and after the negotiation phase and required document submission, the project contract was finally executed in July of 2022:

Award Information
1. Department of Defense Awarding Office: USAMRAA
2. Award number/Project title: W81XWH-22-1-0426 / **SanguiStop: Intravenous Nanomedicine for Targeted Thrombin Delivery in Hemorrhage Control**
3. Type of Award: Grant
4. Type of Award Action: New
5. Project period: July 2022- July 2026

This project is to develop a an intravenously administrable nanoparticle system that can encapsulate thrombin (the enzymatic protein that is generated by coagulation reactions and is responsible for converting fibrinogen to fibrin in hemostasis) and deliver it in a targeted fashion to a bleeding injury site to rapidly form fibrin.

This project has no overlap with PR 191632 (Lyophilized SynthoPlate), but rather is complementary to the PR 191632 since augmenting 'platelets + fibrin' is the ideal goal for rapid hemostatic response.

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.*

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

7. 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.*

8. 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.*