AWARD NUMBER: W81XWH-22-1-0426

TITLE: SanguiStop: Intravenous Nanomedicine for Targeted Thrombin Delivery in Hemorrhage Control

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CONTRACTING ORGANIZATION: Case Western Reserve University

REPORT DATE: AUGUST 2023

TYPE OF REPORT: Annual

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

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
AUGUST 2023	Annual	15JUL2022 - 14JUL2023

4. TITLE AND SUBTITLE

SanguiStop: Intravenous Nanomedicine for Targeted Thrombin Delivery in Hemorrhage Control

5a. CONTRACT NUMBER
W81XWH-22-1-0426

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

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

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Cleveland, Ohio 44106

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: Traumatic hemorrhage remains a primary cause of 'preventable mortality' in austere battlefield conditions for our military. Consequently, the Joint Program Committee-6/Combat Casualty Care. Research Program (JPC-6/CCCRP) emphasizes 'hemorrhage control and resuscitation' as one of the primary focus areas of research towards developing knowledge and materiel solutions for 'on field' mitigation of combat trauma. Robust clinical studies (e.g., PROMMTT, PROPPR, COMBAT and PAMPer), have established that mortality from severe traumatic hemorrhage usually happens within minutes-to-hours post-injury and early intervention via transfusion of whole blood (WB) or its components can significantly improve survival in these patients. While such transfusion strategies are significantly effective in saving lives, implementing them beyond large civilian and military trauma treatment facilities has significant logistical and functional limitations. Among all blood components, platelets play the most critical role in hemorrhage control and yet they present the highest logistical challenges in 'field deployability' and far forward use. Platelets actively stanch bleeding from the injury site by (i) rapidly adhering and aggregating at the bleeding site to form a hemostatic plug (primary hemostasis) and (ii) serving as a procoagulant amplifier by presenting anionic phospholipids, e.g. phosphatidylserine (PS), on the activated platelet surface to render coagulation factor co-localization for tenase and prothrombinase assemblies leading to thrombin burst. The thrombin converts fibrinogen (Fg) to fibrin which then self-assembles and undergoes FXIIIa-mediated crosslinking to secure the hemostatic clot. Consequently, trauma- associated platelet depletion and platelet dysfunction, severely compromise hemostatic function resulting in uncontrolled bleeding. There are tremendous logistical challenges for donor-derived platelet usage in the battlefield, stemming from limited availability and portability, special storage requirements, high risks of bacterial contamination, and very short shelf-life (~5 days at room temperature). Important ongoing approaches to address these challenges are the development and utilization of PRT-platelets, cold-stored platelets (CSP) and freeze-dried platelets (e.g., Thrombosomes by CellPhire), all supported by the DoD. While these approaches can render platelet shelf-life to increase by a couple weeks, these products are still heavily donor-dependent and thus cannot be the stand-alone solutions to address the 'platelet need' for battlefield. This is the technological and logistical gap that the SanguiStop system aims to address. SanguiStop is a thrombin-loaded lipid nanoparticle (LNP) system that can (i) specifically target and accumulate at the injury site via specific binding to von Willebrand Factor (VWF, via VWF-binding peptide or VBP) and collagen (via collagen-binding peptide or CBP), and (ii) directly release thrombin at the site to site-specifically augment/restore fibrin for hemostatic effect, independent of the native platelet status or therapeutic platelet availability.

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
U	U	U	UU	20	19b. TELEPHONE NUMBER *(include area code)*

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1. INTRODUCTION:

Traumatic hemorrhage remains a primary cause of 'preventable mortality' in austere battlefield conditions for our military. Consequently, the Joint Program Committee-6/Combat Casualty Care Research Program (JPC-6/CCCRP) emphasizes 'hemorrhage control and resuscitation' as one the primary focus areas of research towards developing knowledge and materiel solutions for 'on field' mitigation of combat trauma. Robust clinical studies (e.g., **PROMMTT**, **PROPPR**, **COMBAT** and **PAMPer**), have established that mortality from severe traumatic hemorrhage usually happens within minutes-to-hours post-injury and early intervention via transfusion of whole blood (WB) or its components can significantly improve survival in these patients. While such transfusion strategies are significantly effective in saving lives, implementing them beyond large civilian and military trauma treatment facilities has significant logistical and functional limitations. Among all blood components, **platelets play the most critical role in hemorrhage control and yet they present the highest logistical challenges in 'field deployability' and far forward use**. Platelets actively stanch bleeding from the injury site by (i) rapidly *adhering* and *aggregating* at the bleeding site to form a hemostatic plug (*primary hemostasis*) and (ii) serving as a *procoagulant amplifier* by presenting anionic phospholipids, e.g. phosphatidylserine (PS), on the activated platelet surface to render coagulation factor co-localization for *tenase* and *prothrombinase* assemblies leading to *thrombin burst*. The thrombin converts *fibrinogen* (Fg) to *fibrin* which then self-assembles and undergoes FXIIIa-mediated crosslinking to secure the hemostatic clot. Consequently, trauma-associated platelet depletion and platelet dysfunction, severely compromise hemostatic function resulting in uncontrolled bleeding. There are tremendous logistical challenges for donor-derived platelet usage in the battlefield, stemming from limited availability and portability, special storage requirements, high risks of bacterial contamination, and very short shelf-life (~5 days at room temperature). Important ongoing approaches to address these challenges are the development and utilization of PRT-platelets, cold-stored platelets (CSP) and freeze-dried platelets (e.g., Thrombosomes by CellPhire), all supported by the DoD. While these approaches can render platelet shelf-life to increase by a couple weeks, these products are still heavily donor-dependent and thus cannot be the stand-alone solutions to address the 'platelet need' for battlefield. This is the technological and logistical gap that the SanguiStop system aims to address. SanguiStop is a thrombin-loaded lipid nanoparticle (LNP) system that can (i) specifically target and accumulate at the injury site via specific binding to von Willebrand Factor (VWF, via VWF-binding peptide or VBP) and collagen (via collagen-binding peptide or CBP), and (ii) directly release thrombin at the site to site-specifically augment/restore fibrin for hemostatic effect, independent of the native platelet status or therapeutic platelet availability. SanguiStop can become a *complementary* or *adjunctive* intravenous hemostatic therapy that can be administered on-demand at POI and *en route* for hemorrhage control. Beyond its potential use in battlefield conditions, the optimized SanguiStop product can be further translated for hemorrhage control applications in civilian trauma, surgery as well as mass casualty scenarios and resource-limited settings. Therefore, the military (and civilian) relevance of the SanguiStop technology development remains highly significant. The current project is focused on the *in vitro* development and optimization, and *in vitro* as well as *in vivo* evaluation of the SanguiStop nanotechnology as an intravenously administrable platelet surrogate product for point-of-injury 'hemorrhage control'. It is envisioned that this technology will be translated in the future for RDCR application in combat-associated trauma and civilian emergency medical settings, to achieve early hemorrhage control and mitigation of trauma-induced coagulopathies, to save lives.

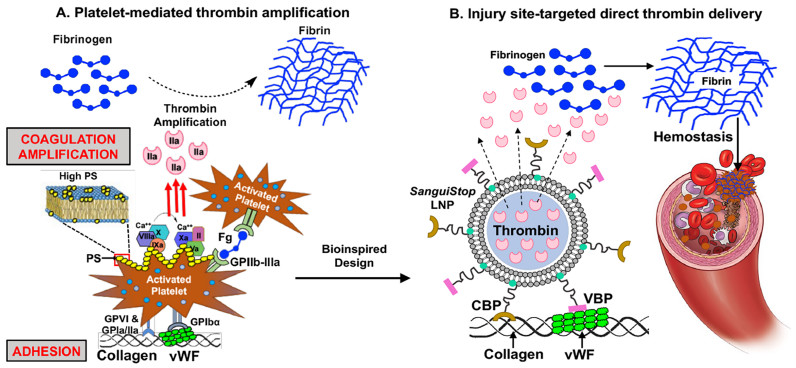
2. KEYWORDS:

Hemorrhage, Trauma, Coagulopathy, Platelets, Coagulation, Thrombin, Fibrin, Hemostasis, Hemorrhage Control, Field-deployable, Intravenous, 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?

The SanguiStop design rationale, envisioned mechanism and hypothesis are stated below:



Central Hypothesis: A lipid nanoparticle system that can be loaded with thrombin and surface-decorated with combination of ligands that bind vWF and collagen, can specifically adhere/aggregate at the vascular injury site, release thrombin by diffusion and phospholipase-triggered LNP destabilization, to site-specifically augment fibrin generation for rapid hemostasis.

The major Specific Aims of the PR211157 project as described in the SOW are:

Aim 1: Evaluate SanguiStop LNPs *in vitro* for hemostatic output using microfluidics and viscoelastometry. The overall focus of this aim is to construct 'vWF + collagen' - targeted SanguiStop LNPs loaded with thrombin and optimize the 'VBP + CBP' decoration density to maximize targeting, as well as, optimize the thrombin loading efficiency and release kinetics to maximize the fibrin generation *in vitro* to enhance clot characteristics under TIC-relevant *simulated* defect/dysfunction settings. The associated sub-aims are:
Aim 1a. Optimize 'VBP + CBP' decoration density on SanguiStop LNP to maximize adhesion on 'vWF + collagen'- coated surface under low-to-high shear flow.
Aim 1b. Optimize thrombin loading efficiency and release kinetics in SanguiStop.
Aim 1c. Evaluate optimized SanguiStop LNP function in Optical Density based fibrin generation assay.
Aim 1d. Evaluate optimized SanguiStop LNP function in microfluidic settings of TIC-relevant clotting defects.
Aim 1e. Analyze SanguiStop LNP effect on clot kinetics and biomechanical characteristics in viscoelastometric settings of TIC-relevant clotting defects.

Aim 2: Evaluate SanguiStop LNPs in vivo for hemostatic efficacy in rat models of trauma and TIC. The overall focus of this aim is to evaluate the *in vitro* optimized best-performing SanguiStop LNPs *in vivo* in validated rat models of TIC, including pharmacokinetics, safety and hemostatic efficacy. The associated subaims are:
Aim 2a. Study pharm/tox and biodistribution profile of SanguiStop in uninjured rats.
Aim 2b. Evaluate hemostatic efficacy of SanguiStop LNPs in a rat model of single acute hemorrhagic trauma.
Aim 2c. Evaluate hemostatic efficacy of SanguiStop LNPs in a rat model of polytrauma TIC.

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 In Year 1 (Fall 2022- Fall 2023):

Aim 1a. Optimize 'VBP + CBP' decoration density on SanguiStop LNP to maximize adhesion on 'vWF + collagen'- coated surface under low-to-high shear flow.

VBP (Cysteinylated peptide sequence CTRYLRIHPQSWVHQI) and CBP (Cysteinylated peptide sequence C-$[GPO]_7$) were obtained commercially (from GenScript) and reacted with 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[maleimide(polyethylene glycol)$_{2000}$ (DSPE-PEG$_{2K}$-Mal), to render reaction between the thiol (-SH) terminus of the peptide sequences and the Maleimide terminus of the lipid-PEG molecules to for succinimidyl thioether conjugates (reaction schematic shown in **Figure 1A**). The resultant DSPE-PEG$_{2K}$-peptide conjugates were combined with distearoylphosphatidylcholine (DSPC) and cholesterol (Chol) at various controlled mole% ratios, and subjected to think-film rehydration followed by extrusion methodology (process schematic shown in **Figure 1B**) to yield lipid nanoparticles (LNPs) bearing various densities of 'VBP + CBP' motifs.

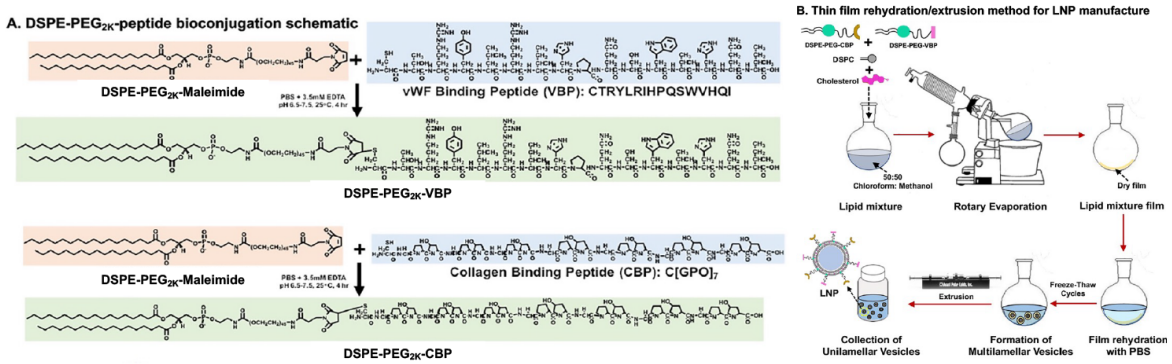


Figure 1. A: Maleimide-Thiol based bioconjugation reaction schematic to synthesize DSPE-PEG2K-VBP and DSPE-PEG2K-CBP molecules. **B:** Process schematic to manufacture LNPs heteromultivalently decorated with VBP and CBP.

The conjugation reactions of VBP and CBP to DSPE-PEG$_{2K}$-Mal to form DSPE-PEG$_{2K}$-VBP and DSPE-PEG$_{2K}$-CBP were confirmed by matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry. Representative data are shown in **Figure 2**.

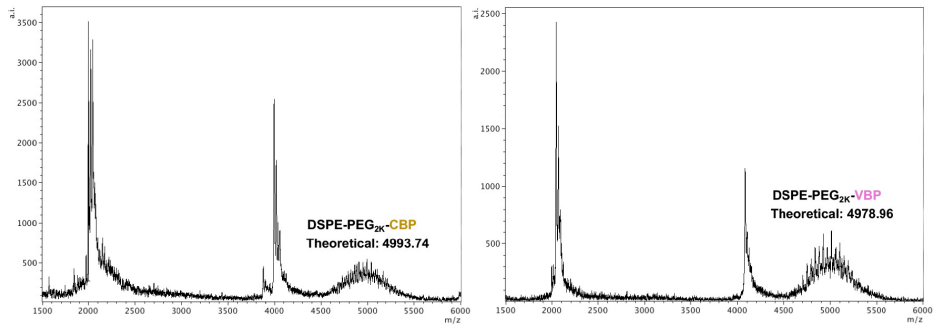


Figure 2. Representative MALDI-TOF mass spec data for DSPE-PEG$_{2K}$-peptide conjugates.

Resultant manufactured LNPs were characterized by dynamic light scattering (DLS) and cryo-transmission electron microscopy (cryo-TEM) for size and morphology. Table 1 shows representative DLS for the manufactured LNPs for various peptide (VBP and CBP) content.

Total Peptide mole %	CBP:VBP	Hydrodynamic diameter (nm)	Number average diameter (nm)	PDI	Zeta potential (mV)
0 (control)	-	195.47	151.94	0.125	-18.1 ± 1.8
1.25%	100:0 (CBP only)	196.64	151.66	0.054	-27 ± 1.9
	0:100 (VBP only)	180.14	116.02	0.227	-20.9 ± 1.5
	50:50 (Dual targeting)	199.91	156.19	0.134	-24.5 ± 1.3
2.5%	100:0 (CBP only)	191.4	145.97	0.158	-32.4 ± 1.9
	0:100 (VBP only)	224.7	151.29	0.227	-23.4 ± 0.7
	50:50 (Dual targeting)	217.8	143.53	0.197	-24.5 ± 1.3
	75:25 CBP:VBP	215.1	154.64	0.243	-24.8 ± 1.3
	25:75 CBP:VBP	217.9	149.05	0.173	-24.2 ± 1.6
5%	100:0 (CBP only)	213.1	164.9	0.003	-32.5 ± 2.3
	0:100 (VBP only)	-	-	-	-
	50:50 (Dual targeting)	657	135.75	0.349	-24.6 ± 0.9

Table 1: Representative DLS and Zeta Potential data for LNPs with varying VBP: CBP content

As evident from the representative data, irrespective of VBP/CBP mole % the LNPs have ~ 150 nm diameter and approximately –20 mV zeta potential for the 'VBP + CBP'-decorated particles, consistently. The unilamellar vesicle morphology of the LNPs were characterized by cryo-TEM. Representative cryo-TEM image of vesicle morphology is shown in **Figure 3:**

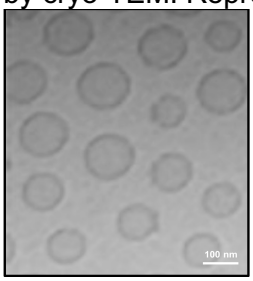


Figure 3. Representative cryo-TEM image of peptide-decorated LNP vesicles showing unilamellar spherical morphology and a size distribution in the 100-150 nm diameter range.

The resultant LNPs were evaluated in a BioFlux microfluidic system (Fluxion Biosciences) to assess adhesion capability to 'VWF + collagen'-coated microfluidic channel, and optimize peptide decoration density and composition to maximize the adhesion and stable anchorage to this 'VWF + collagen'- coated surface. The BioFlux setup is shown in **Figure 4**:

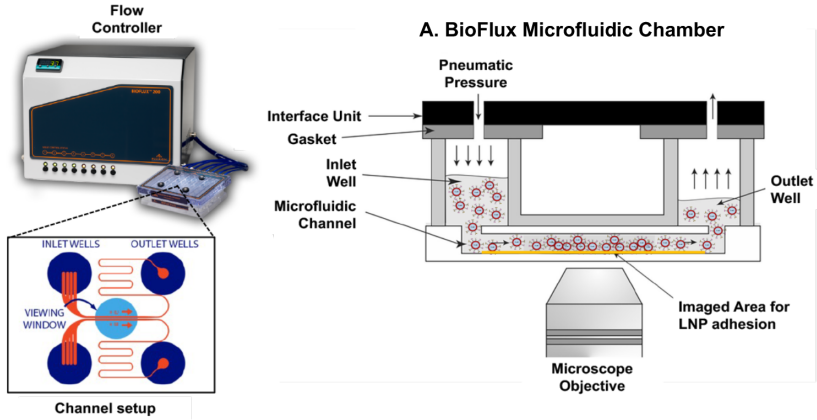


Figure 4. Schematic of BioFlux system: The 'flow controller' allows pneumatic control of flow rate of the 'inlet fluid' to maintain various shear flow conditions in the channel; Channel surface is coated with collagen, and red fluorescent LNPs along with 5 µg/ml soluble VWF is introduced for flow through channel and LNP adhesion is imaged in real time.

As shown in the BioFlux setup description, the adhesion of red fluorescent LNPs was imaged in real time for 10 min flow-period per experiment. Kinetics of red fluorescence intensity increasing in real time was considered as reflective of 'LNP adhesion kinetics', and the end point image at the 10-min timepoint after washing away loosely bound particles with buffer, was considered as the 'final image of adhered particles' for each experiment. **Figure 5** below shows representative endpoint images of LNPs bearing 2.5 mole % total peptide (VBP only, CBP only, or 'VBP + CBP' combination at 1:1 ratio) at low (5 dyn/cm^2), medium (30 dn/cm^2) and high (60 dyn/cm^2) shear flow conditions.

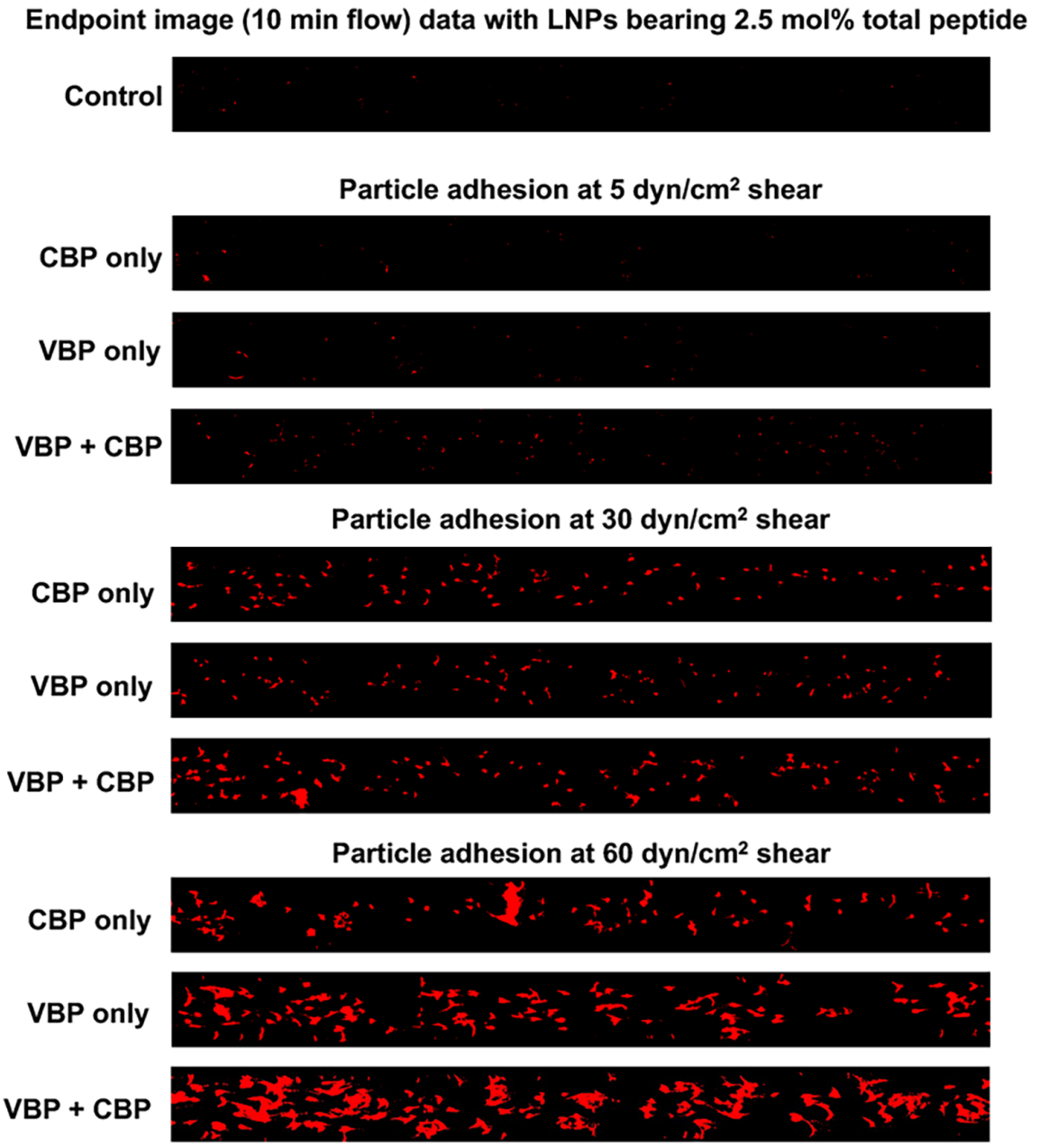


Figure 5. Representative BioFlux endpoint (after 10 min flow period for each experiment) images of LNP adhesion on 'VWF + collagen'-coated surface, for LNPs bearing 2.5 mole% total peptide and flowed at low, medium, and high shear flow conditions.

Figure 6 below shows the adhesion kinetics analysis and the endpoint surface coverage analysis of red fluorescent LNPs bearing 2.5 mole% total peptide content, flowed in the BioFlux microfluidic channels at low (5 dyn/cm2), medium (30 dyn/cm2) and high (60 dyn/cm2) shear conditions.

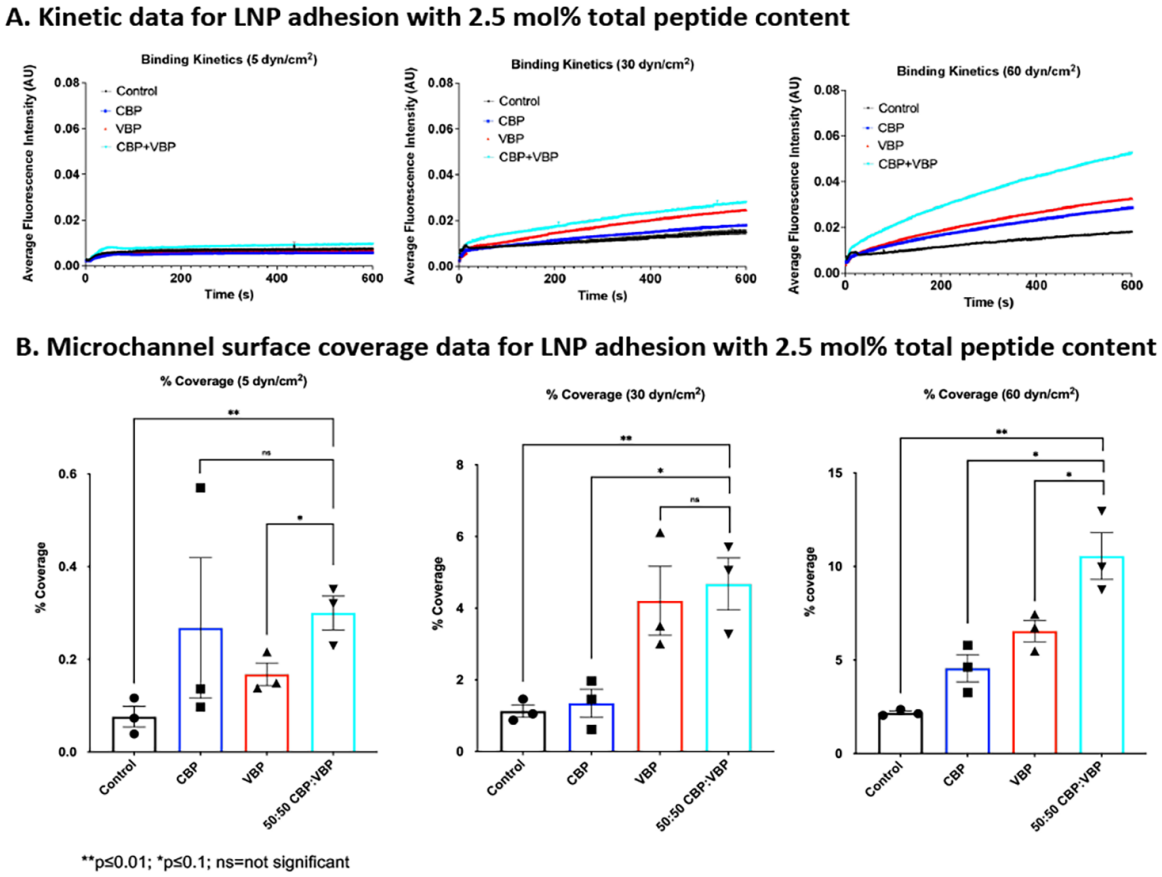


Figure 6: A: Kinetic data for LNP adhesion and **B:** Endpoint analysis of LNP surface coverage for LNPs bearing 2.5 mole % total peptide (VBP only, CBP only or VBP + CBP) flowed in microfluidic channels over collagen-coated surface in presence of 5 µg/ml soluble VWF.

As evident from the representative results shown in **Figure 5** and **Figure 6**, across all shear conditions LNPs bearing 'VBP + CBP' combination decoration at 1:1 ratio and total 2.5 mole% peptide content shows higher surface adhesion and coverage compared to LNPs bearing VBP-only or CBP-only decoration. Additionally, the adhesion kinetics and surface-coverage of the 'VBP + CBP'-decorated LNPs (2.5 mole % total peptide content) seems to increase at higher shear. It is well-established in literature and hematology research that VWF unraveling to expose its biointeractive domains is shear-sensitive (occurs at higher shear) and therefore VWF assembly on collagen (via VWF A3 domain) can also be expected to increase at higher shear conditions. Therefore, our data suggests that there may be higher VWF unraveling and assembly on collagen, thereby increasing the availability of VWF substrate on the microfluidic channel surface and this may be enabling higher binding of the LNPs via the VBP motif in addition to the collagen binding via the CBP motif. In fact, even the VBP-only LNPs (red line in Figure 6A and red bar in Figure 6B) seem to undergo enhanced adhesion kinetics and surface-coverage as shear condition increases, and this validates the 'higher VWF binding by VBP at higher shear' hypothesis. Overall, the 'VBP + CBP' combination decorated LNPs undergo higher anchorage and retention (hence surface-coverage) on 'VWF + collagen' surface at higher shear.

<u>Aim 1b.</u> Optimize thrombin loading efficiency and release kinetics in SanguiStop.

Thrombin loading studies in the LNPs were carried out by adding a thrombin solution (in buffer) during the thin film rehydration step of the LNP manufacture (please refer back to **Figure 1B** for the LNP manufacturing steps). Specifically, the lipid films were generated by subjecting a mixture of DSPE-PEG-peptide conjugates, DSPC and cholesterol (total 2.5 mole% of DSPE-PEG-VBP and DSPE-PEG-CBP at 1:1 ratio used as starting metric) in methanol: chloroform (50:50) to rotary evaporation under reduced pressure in a round bottom flask. The resultant thin lipid film was rehydrated with a solution of 500 nM human alpha thrombin in Tris Buffered saline (TBS), sonicated for 30 minutes, and then subsequently extruded five times through a 200 nm pore size polycarbonate filter to yield thrombin-loaded 'VBP + CBP'-decorated LNPs. These LNPs were passed through a column packed with TBS-swelled G-100 Sephadex beads, to remove unencapsulated thrombin, and the isolated thrombin-loaded LNPs were characterized for loading efficiency and release kinetics. For loading efficiency, total thrombin loading was determined by adding a 1:1 mixture of methanol:chloroform to the thrombin-loaded LNPs to induce complete particle dissolution and exhaustive release of thrombin, and this exhaustively released thrombin amount was determined using a Human thrombin ELISA assay (Abcam, USA). The release kinetics was measured by sealing the column separated thrombin-loaded LNPs in a 100K MWCO dialysis bag, placing the bag in a TBS reservoir, and removing aliquots from the reservoir at pre-defined timepoints over the course of six hours (fresh TBS added back to reservoir appropriately after each aliquot removal). To compare this diffusive release kinetics of thrombin from the LNPs to enzyme (e.g., sPLA2) triggered release kinetics, similarly prepared thrombin-loaded LNPs were mixed with 25 µg/ml $sPLA_2$, sealed in dialysis tubing and subjected to similar analysis. Thrombin concentrations from collected aliquots in the above-described release mechanism conditions were evaluated using the Human thrombin ELISA assay and plotted over time to determine release profile. **Figure 7** shows preliminary results from these studies, with **7A** demonstrating reproducible loading of thrombin in the LNPs and **7B** demonstrating that enzyme-triggered release mechanism results in higher and faster release of thrombin from the LNPs, compared to diffusive release mechanism.

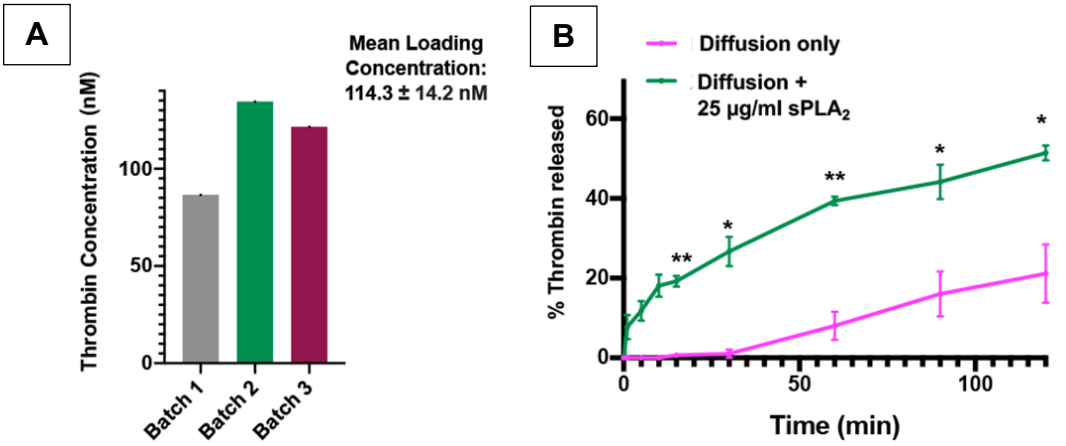


Figure 7. A: Representative data showing effective thrombin loading in the LNPs in the 100-130 nM range; **B:** Diffusive release mechanism results in slow release of ~ 20% of the loaded thrombin from the LNPs over a 2-hour period, while LNP destabilization triggered by the sPLA2 results in almost immediate release of the thrombin, reaching ~ 60% release over the same 2-hour period.

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 1 total):

Protocol [HRPO Assigned Number]: **To be approved and assigned.**

Title: **In Vitro Blood Analysis with Hemostatic Agent Delivering Nanoparticles**

Target required for clinical significance: N/A as this is for in vitro analysis only, with blood from healthy blood donors that will be manipulated in vitro for inducing hemostatic defects and treated with hemostat-loaded nanoparticles (e.g. thrombin-loaded SanguiStop LNPs) for evaluating treatment effects in vitro using microfluidics, biochemical assays, chromogenic/fluoresgenic assays and viscoelastometric assays.

Target approved for clinical significance:

Submitted to and Approved by: Case Western **IRB approved on December 23, 2022**. OHRO/HRPO documents submitted for review.

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

Status: OHRO/HRPO approval pending

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.

(b) Use of Human Cadavers for Research Development Test & Evaluation (RDT&E), Education or Training: NOT APPLICABLE to this project

"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:

- *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):

2 Protocols at CWRU: Single injury in rats, and Polytrauma injury in rats, pending IACUC approval at CWRU.

Rat studies for PR211157 starts in Aim 2 (In Year 3 as per SOW). Therefore, we will obtain IACUC approval at CWRU in Year 2 and submit to DOD for ACURO approval.

PROTOCOL(S): PENDING

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

The project allowed enhancing the DOD-supported research endeavors of the PI, Dr. Anirban Sen Gupta, to be able to advance intravenous hemostatic nanotechnologies for on-field hemorrhage control in combat casualty care. The project enabled training and professional development opportunity for two post-doctoral researchers (Dr. Bipin Paruchuri and Dr. Rohini Sekar), one research engineer technician (Hanyang Wang), and several undergraduate researchers (Jenny Lian, Danielle Sun) in the area of biomedical engineering specifically focused on hemostatic nanotechnologies. These research personnel got mentored and trained by Dr. Sen Gupta in the theoretical aspects of blood coagulation, hemostasis and TIC, and experimental methodologies of nanoparticle manufacture and characterization, microfluidics, fluorescence imaging, thrombin and fibrin generation assays, and statistical data analyses. Several of these research personnel will be presenting their research at MHSRS and other meetings in 2024.

How were the results disseminated to communities of interest?

Nothing to Report for Year 1.
Results for Year 1 studies are currently being expanded with statistical volume of data, to enable submission of manuscript to peer reviewed journal in early 2024. We have previously published our research on hemostatic nanotechnologies in Journal of Trauma and Acute Care Surgery, Journal of Thrombosis and Haemostasis, Blood, Biomaterials, Bioconjugate Chemistry, Biomacromolecules, ACS Nano and Science Translational Medicine. Thus we anticipate submitting manuscripts to such high impact peer-reviewed journals. We also anticipate presenting our research at Hemostasis Gordon Research Conference, MHSRS, ISTH etc. national and international meetings in 2024.

During Year 2, we plan to complete ALL SUBTASKS pertaining to Aim 1:

Aim 1a. ***Optimize 'VBP + CBP' decoration density on SanguiStop LNP to maximize adhesion on 'vWF + collagen'- coated surface under low-to-high shear flow.***
Aim 1b. ***Optimize thrombin loading efficiency and release kinetics in SanguiStop.***
Aim 1c. ***Evaluate optimized SanguiStop LNP function in Optical Density based fibrin generation assay.***
Aim 1d. Evaluate optimized SanguiStop LNP function in microfluidic settings of TIC-relevant clotting defects.
Aim 1e. ***Analyze SanguiStop LNP effect on clot kinetics and biomechanical characteristics in viscoelastometric settings of TIC-relevant clotting defects.***

As described in our experiment data and achievement sections above, during Year 1 we have made considerable progress with Aim 1a and Aim 1b. We will continue to expand on these subtasks to complete the statistical volume of studies to establish our optimized thrombin-loaded LNP system.

For Aim 1c, 1d and 1e, we will be requiring the use of human blood and plasma, and therefore we anticipate that upon OHRO/HRPO approval of our approved IRB protocol to collect blood from volunteer donors, we will initiate, carry out and complete these aims regarding 'treatment effect characterization' of the thrombin-loaded SanguiStop LNPs in human blood/plasma samples that are in vitro manipulated (e.g. platelet-depleted, anticoagulant-treated, fibrinolytic-treated etc.) to create various 'hemostatic compromise' scenarios that could be potentially treated by SanguiStop for hemostatic rescue.

We will also submit our IACUC documents for ACURO approval during the third quarter of Year 2, such that by the end of Year 2 we can have ACURO approval to potentially initiate our in vivo model studies in early Year 3, as planned in our SOW. These studies pertain to the Aim 2 of the proposal.

4. IMPACT:

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

The main impact of Year 1 studies is the advancement of an intravenous hemostatic nanoparticle platform that can enable direct thrombin delivery and release in an injury-site targeted enzyme-responsive fashion to potentially restore fibrin status for hemostatic effect. This system can potentially restore hemostatic outcomes independent of native platelet status and therapeutic platelet transfusion availability status. Injury site-localized delivery of thrombin (alone or with fibrinogen) has well-demonstrated efficacy in externally accessible or topical hemostatic applications (e.g. fibrin glue, wound dressings etc.), but an intravenous system allowing systemic administration of thrombin while maintaining systemic safety and injury site-targeted mechanism of action, is yet to be established in hemostatic applications. Our Year 1 studies move the technology towards this innovative application potential. Translational advancement of this system, by virtue of completing Aim 1 and Aim 2 milestones as delineated in our SOW, can provide unique capabilities for far forward combat casualty care where blood products to mitigate traumatic hemorrhage complications are in limited availability.

What was the 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 technologies that are easily portable in pre-hospital settings, to help management of various pathological conditions. The findings also provide methodological insight into evaluation of manufacturing process, safety and efficacy of drug-delivering nanoparticle systems in general.

What was the impact on technology transfer?

Our research is anticipated to lead to potential new patents in hemostatic nanotechnologies.

What was the impact on society beyond science and technology?

Nothing to report in Year 1.
As we anticipate publications and conference presentations in Year 2 onwards, we envision improving public knowledge, skills, and healthcare capabilities, especially in the area of trauma care.

5. CHANGES/PROBLEMS:

Nothing to Report

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

Nothing to Report.

Changes that had a significant impact on expenditures

N/A

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

Significant changes in use or care of human subjects

N/A

Significant changes in use or care of vertebrate animals

N/A.

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:

Nothing to Report in Year 1.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

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, training and mentoring regarding experimental design, execution and analysis for studies proposed under the Specific Aim 1 of the project, in Year 1. Dr. Sen Gupta also prepared and submitted technical progress report.

Bipin Paruchuri, PhD
Project Role: Post Doctoral Research Associate at CWRU in Sen Gupta lab
Research Identifier:
Nearest person month worked: 12
Contribution to Project: Dr. Paruchuri contributed to LNP manufacture, characterization and microfluidic analysis.

Rohini Sekar, PhD
Project Role: Post Doctoral Research Associate at CWRU in Sen Gupta lab
Research Identifier:
Nearest person month worked: 12
Contribution to Project: Dr. Sekar contributed to LNP manufacture, characterization and thrombin loading/release studies.

Hanyang Wang, BS
Project Role: Research Engineer Technician at CWRU in Sen Gupta lab
Research Identifier:
Nearest person month worked: 12
Contribution to Project: Mr. Yang assisted Dr. Paruchuri and Dr. Sekar in conducting replicates of the various studies (microfluidics, thrombin loading/release) pertaining to Aim 1 of the project.

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

The following new grants were awarded to the PI (Sen Gupta) during this reporting period but this has not altered his role and effort in PR 211157:

1. DoD PRMRP IIRA PR201584 Sen Gupta (Co-I) 09/2021- 08/2024

Total direct:

TraumaChek^{TM}: 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. 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 PR 211157 but are rather complementary to the milestones of PR 211157, 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?

Nothing to Report.
The SanguiStop project is 100% conducted at CWRU.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: *N/A*

QUAD CHARTS: *N/A*

9. APPENDICES:

N/A.