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TITLE: SynthoPlate Nanotechnology for Intravenous Hemostasis and Wound Healing in Prolonged Field Care

PRINCIPAL INVESTIGATOR: Anirban Sen Gupta

CONTRACTING ORGANIZATION: Case Western Reserve University, Cleveland, OH

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14. ABSTRACT

Combat trauma-associated uncontrolled hemorrhage and coagulopathy remain the leading causes of morbidity and mortality in the military. Overwhelming evidence from military based resuscitation studies has indicated that platelet transfusion can significantly reduce these events in prolonged field care scenarios. However, platelet transfusion suffers from unique logistical and functional challenges in a far forward military setting, due to (i) limited availability and portability of platelet concentrates, (ii) special storage requirements to minimize platelet activation and granulation, (iii) high risk of bacterial contamination and (iv) very short shelf-life (3-5 days). Furthermore, blood type compatibility issues can limit early intervention. Other platelet-derived products, e.g., frozen (-80C), cold-stored (4C) or lyophilized platelets and platelet membrane-derived vesicle technologies (e.g. Infusible Platelet Membrane and Thrombosome) may suffer from similar limitations and performance variabilites. These challenges have led to robust research efforts for creating a shelf-stable, highly portable, readily deliverable 'platelet substitute' that can mimic platelet-mediated mechanisms of hemostasis, while avoiding systemic immunogenicity and off-target harmful effects. To this end, we have created a lipid-peptide conjugate based synthetic platelet technology (SynthoPlateTM, US patent 9107845, TRL 4), that mimics the inherent platelet-mediated mechanisms of primary and secondary hemostasis in a bleeding site-selective fashion, without presenting systemic risks.

15. SUBJECT TERMS
None listed.

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TABLE OF CONTENTS

	Page
1. Introduction	4
2. Keywords	4
3. Accomplishments	4
4. Impact	10
5. Changes/Problems	11
6. Products	11
7. Participants & Other Collaborating Organizations	11
8. Special Reporting Requirements	13
9. Appendices	13

1. Introduction: Combat trauma-associated uncontrolled hemorrhage and coagulopathy remain the leading causes of morbidity and mortality in the military. Overwhelming evidence from military based resuscitation studies has indicated that platelet transfusion can significantly reduce these events in prolonged field care scenarios. However, platelet transfusion suffers from unique logistical and functional challenges in a far forward military setting, due to (i) limited availability and portability of platelet concentrates, (ii) special storage requirements to minimize platelet activation and granulation, (iii) high risk of bacterial contamination and (iv) very short shelf-life (3-5 days). Furthermore, blood type compatibility issues can limit early intervention. Other platelet-derived products, e.g., frozen (-80C), cold-stored (4C) or lyophilized platelets and platelet membrane-derived vesicle technologies (e.g. Infusible Platelet Membrane and Thrombosome) may suffer from similar limitations and performance variabilities. These challenges have led to robust research efforts for creating a shelf-stable, highly portable, readily deliverable 'platelet substitute' that can mimic platelet-mediated mechanisms of hemostasis, while avoiding systemic immunogenicity and off-target harmful effects. To this end, we have created a lipid-peptide conjugate based synthetic platelet technology (SynthoPlate^{TM}, US 9107845, TRL 4), that mimics the inherent platelet-mediated mechanisms of primary and secondary hemostasis in a bleeding site-selective fashion, without presenting potential systemic risks. In the current project, we seek to evaluate the point-of-care hemostatic efficacy and spatio-temporally targeted wound healing treatment applicability of the SynthoPlate^{TM} nanotechnology in appropriate porcine models, with a vision to translate this technology for prolonged combat casualty care in a far forward setting. Our specific aims are:
Aim 1. Characterization of biodistribution, systemic risks and immune response of intravenously administered SynthoPlate^{TM} in pigs.
Aim 2. Evaluation of hemostatic efficacy of pristine SynthoPlate^{TM} and TXA-loaded SynthoPlate^{TM} in a pig model of polytrauma.
Aim 3. Evaluate the efficacy of SynthoPlate^{TM} alone or in combination with Gentamicin to provide wound protection and improve re-epithelialization in porcine wound models.

2. Keywords: Trauma, Hemorrhage, Burn, Wound, Transfusion, Platelets, Synthetic Platelets, TXA, Intravenous, Hemostasis, Pig Model

3. Accomplishments:

Year 1(Months 1-12)

As per the proposed SOW, the Year 1 Aims and Major Goals were:

Aim 1. Characterization of biodistribution, systemic risks and immune response of intravenously administered SynthoPlate^{TM} in pigs.

Major Task 1: Characterize and Mitigate immune response (if any) to SynthoPlate dosing
Major Task 2: Characterize prothrombotic risks (if any) upon SynthoPlate administration
Major Task 3: Characterize biodistribution of SynthoPlate over time.
Major task 4: Establish a safe dosing protocol for SynthoPlate in pigs.

Majority of these tasks were completed in Year 1, except for histopathology of pig organs which were slightly delayed and extended into Year 2. This was because the 'histopathology kits' were on 'backorder'. Nonetheless, they were ultimately completed in early Year 2. Year 1 annual report was submitted in November 2018. Components of results stemming from Year 1 studies were included in a manuscript titled "***Intravenous synthetic platelet (SynthoPlate^{TM}) nanoconstructs reduce bleeding and improve 'golden hour' survival in a porcine model of traumatic arterial hemorrhage***", that was published in *Nature Scientific Reports* (Vol 8, Article Number: 3118, 2018). Some of the results were also part of a recent presentation on the SynthoPlate^{TM} nanotechnology that was given by Dr. Anirban Sen Gupta (PI) to NAMRU San Antonio as part of the ORISE program. Some of the results were also exhibited as part of a poster presented in the Hemorrhage Control program at MHSRS 2018.

Aim 2. Evaluate hemostatic efficacy of pristine SynthoPlate^{TM} and TXA-loaded SynthoPlate^{TM} in porcine model of polytrauma.

Major Task 1: Demonstrate that post-injury SynthoPlate transfusion results in a significant reduction in blood loss following polytrauma

- Subtask 1: SynthoPlate manufacture and sterilization for shipment to UPitt
- Subtask 2: IACUC and ACURO approval at UPitt
- Subtask 3: Sham/Control studies in pig trauma model

SynthoPlate manufacture and sterilization was completed. IACUC and ACURO approval for pig model studies in Neal lab was approved at UPitt: DM160354.04 entitled, "Exploring Dynamic Platelet Functions after Hemorrhagic Shock, Polytrauma, and Associated Coagulopathies in Swine," IACUC protocol number 17110765, Protocol Principal Investigator Matthew Neal. Sham/control studies in pig were initiated.

Aim 3: Evaluate the efficacy of SynthoPlate alone or in combination with Gentamicin to provide wound protection and improve re-epithelialization in porcine wound models.

Major Task 1: Demonstrate that SynthoPlate alone has beneficial effect on wound protection and inflammation.

- Subtask 1: CRADA establishment with Dr. Chan's lab, USAISR

CRADA was established with Dr. Chan's lab at ISR, accordingly.

Year 2 (Months 12-24)

As per the proposed SOW, the Year 2 Aim and Major Goals were:

Aim 2. Evaluate hemostatic efficacy of pristine SynthoPlate^{TM} and TXA-loaded SynthoPlate^{TM} in porcine model of polytrauma.

Major Task 1: Demonstrate that post-injury SynthoPlate transfusion results in a significant reduction in blood loss following polytrauma

- Subtask 3: Continuation of studies with sham/control pig model
- Subtask 4: Evaluation of hemostatic effect and serum cytokines in SynthoPlate-dosed pig traumatic injury model

Major Task 2: Evaluate the potential synergistic effect of SynthoPlate and site-specific delivery of TXA on blood loss and hemodynamic changes after injury.

- Subtask 1: Manufacture and characterization of SynthoPlate loaded with TXA

All pig model studies were conducted as per IACUC and ACURO approval for Neal lab studies at UPitt: DM160354.04 entitled, "Exploring Dynamic Platelet Functions after Hemorrhagic Shock, Polytrauma, and Associated Coagulopathies in Swine," IACUC protocol number 17110765, Protocol Principal Investigator Matthew Neal. New personnel (a new animal surgeon) was added to Neal Lab IACUC during Quarter 1 of Year 2. The actual study method and assessment criteria were not altered at all, and hence we did not anticipate that this would be considered 'non-compliance' in ACURO framework. We received a letter of noncompliance for a protocol violation during the Quarter 2. When advised of the noncompliance, the new individual suspended participation until official approval was available, and we delayed additional studies until the protocol was fully reviewed and approval granted, effective 07-MAR-2019. Upon approval of ACURO, we resumed swine studies of uncontrolled hemorrhage at the University of Pittsburgh. In our studies, the post-injury administration of SynthoPlate in the pig trauma model showed transient hemodynamic changes (tachycardia) and oxygen desaturations following the dose administration, along with transient coloration of the skin. We proceeded to investigate whether this is a result of the particles themselves, or whether this is related to a species (pig)-specific pulmonary macrophagic or innate immune response (e.g. see *Skotland, T. Theranostics 2017; 7(19): 4877–4878*.) or complement-mediated pseudo-allergic response (CARPA) that have been reported regarding some nanoparticle formulations in pigs previously by other researchers (e.g. see *Szebeni J. Mol Immunol 2014; 61: 163-173, PMID: 25124145*). Our observations led us to investigate the combinations of various peptide components (VBP, CBP and FMP) decorated on SynthoPlate and test for specific peptide interactions as a source of the cardiopulmonary changes. To this end, we collected plasma for a planned analysis of cytokine expression by Luminex. We also measured complement activation levels before and immediately following injury utilizing ELISA kits for C3a and C5a to assess CARPA as an etiology. The details of our studies and results are described in Year 2 Annual Report. From our studies it seems that systemic CARPA is not an issue in the pigs, with our control or SynthoPlate particles. It is important to note here that we also saw that complement activation was not an issue in our uninjured pigs and pilot studies of femoral artery bleeding model in pigs that we carried out prior to the current PFCRA-funded studies and published in *Nature Scientific Reports*. As a result, we conjectured that the reaction we are seeing is most possibly a species-specific reaction reported for pigs and other *cloven-hoofed animals* that have a special type of macrophages called Pulmonary Intravascular Macrophages (PIMs) that are sensitive to any nanoparticle (*Skotland, T. Theranostics 2017; 7(19): 4877–4878).* To further test this, we decided to carry out studies with the ex vivo analysis of pig lung macrophages. We also wanted to study the cardiopulmonary distress aspect in real time using ultrasound imaging in pigs. These approaches required us to modify the UPitt IACUC with amendments to allow these studies and then submit the amended

IACUC for further ACURO approval. Our thought process was if we confirm the species-specific PIM to be culprit regarding the cardiopulmonary reaction seen in pigs, then we will either request an evidence-based amendment to our IACUC or ACURO to allow pre-treatment based depletion of these PIMs in pigs (e.g. by pre-treatment dosing of indomethacin or clodronate-loaded liposomes), or if needed, then request a change of species in our model (most possibly to rabbits), for subsequent studies in the future. As for *Major Task 2 Subtask 1*, we successfully loaded TXA in SynthoPlate and characterized its encapsulation and release. The details of our studies have been incorporated in Annual Report of Year 2, and part of the TXA encapsulation work was included in a recent publication (*Girish et al., J Thromb Haemost, 2019; 17: 1632-1644*).

Aim 3: Evaluate the efficacy of SynthoPlate alone or in combination with Gentamicin to provide wound protection and improve re-epithelialization in porcine wound models.

Major Task 1: Demonstrate that SynthoPlate alone has beneficial effect on wound protection and inflammation.

- Subtask 2: Shipment of SynthoPlate (SP) and Gentamicin-loaded SP to Chan lab
- Subtask 3: Studies with localized application of SynthoPlate on burn wound in pigs for healing efficacy
- Subtask 4: Report development on Major Task 1

Unloaded SynthoPlate (SP) and Gentamicin-loaded SynthoPlate (Genta-SP) were adequately characterized in Sen Gupta lab and shipped to Chan lab for pig burn model studies. For these studies, 32 9cm^2 deep partial thickness burns were made on the dorsum of a pig. In an effort to keep each treatment localized to the appropriate wound, a 2-layered piece of gauze cut to the size of the wound was secured to each site with tegaderm. The appropriate treatment – normal saline, gentamicin, SynthoPlate, or Genta-SynthoPlate was applied to each wound. A larger tegaderm was then used over all the wounds to further secure the treatments, and finally loban was used to cover the entire back. Unfortunately, despite efforts to sequester each of the treatments, upon return to the operating room one week later, it was clear that the treatments had not remained on their individual wounds but may have instead spread throughout. Nonetheless, the SP and Genta-SP group showed some promising trend in healing, as described in detail in the Annual Report of Year 2. In the context of avoiding treatments running out of the wound area, we planned to revise the wound model set-up for the subsequent experiments in two ways: (1) Create only 16 wounds with more distance between each wound and (2) Sequestering the treatments to their appropriate wounds by platform wound devices used in other experiments by the Chan group. The plan was to follow the wounds out to 90 days as opposed to 28 to also examine re-epithelialization and scarring in the same animals. The new device set-up was described in Annual Report of Year 2, and IACUC amendments to reflect these studies were submitted for approval, with the hope of subsequent ACURO approval to carry out the studies in Year 3.

Year 3 (Months 24-36)

Aim 2. Evaluate hemostatic efficacy of pristine SynthoPlate™ and TXA-loaded SynthoPlate™ in porcine model of polytrauma.

Major Task 1: Demonstrate that post-injury SynthoPlate transfusion results in a significant reduction in blood loss following polytrauma

- Subtask 4: Evaluation of hemostatic effect and serum cytokines in SynthoPlate-dosed pig traumatic injury model (after establishing the mechanism behind the transient cardiopulmonary reaction seen in injured pigs upon nanoparticle dosing)

Major Task 2: Evaluate the potential synergistic effect of SynthoPlate and site-specific delivery of TXA on blood loss and hemodynamic changes after injury.

- Subtask 2: Evaluate 'control particle + TXA' vs 'SynthoPlate + TXA' in pig trauma model

Major task 3: Identify effects of TXA additively mixed vs encapsulated in SP vesicle on organ injury and inflammation following trauma

Major task 4: Data analysis and report preparation on Sp Aim 2.

For Aim 2 swine studies, the model involves the use of male and female Yorkshire swine (30-35kg). As described in the Final Report of Year 2, the post-injury administration of SynthoPlate in the pig trauma model showed transient hemodynamic changes (tachycardia) and oxygen desaturations following the dose administration, along with transient coloration of the skin. Therefore, we focused on studying the potential mechanism behind these observations. This required an amendment to our IACUC protocol, to enable the use of a bronchoalveolar lavage (BAL) to collect macrophages, Swan-Ganz catheterization , and ultrasound imaging of the pig, as well as addition of another personnel (Hernando Gomez) who could assist with these envisioned studies. The IACUC amendment request was submitted by Dr. Neal's (Co-I at UPitt) laboratory, and once the amendment was approved by UPitt IACUC, the revised IACUC was submitted for ACURO approval. No animal studies were performed under DM160354 award during this time while the IACUC protocol was under ACURO evaluation. The ACURO approval was obtained during February 2020. However the studies could not be performed since all laboratories were closed due to COVID-19 related shutdowns and stay-at-home orders that started in March 2020.

Aim 3: Evaluate the efficacy of SynthoPlate alone or in combination with Gentamicin to provide wound protection and improve re-epithelialization in porcine wound models.

Major Task 1: Demonstrate that SynthoPlate alone has beneficial effect on wound protection and inflammation.

Major Task 2: Demonstrate SynthoPlate-Gentamicin combinations provide wound protection and decrease inflammation by decreasing the bacterial burden and local inflammatory markers.

Major Task 3: Demonstrate SynthoPlate alone or with Gentamicin combination has a beneficial effect on re-epithelialization of a wound

Major Task 4: Final report generation

The animal use protocol associated for these studies, entitled, "A Validation Study of Scarring After Skin Loss Either as a Result of Trauma or Burns in a Porcine Animal Model - A Type Protocol," required amendments for procedural modifications as described in the Annual Report of Year 2. REASON FOR CHANGE(S): A-14-042-TS4 has always been intended to be an extension of the A-14-041-TS4 protocol. The A-14-042-TS4 protocol was written to be the large wound version of A-14-041-TS4. Although this treatment was initially applied to the wounds

described in A-14-041-TS4, we found that the wounds were too small (3cm diameter) and close together and that the dressing applied was unable to keep the treatments contained to the wound they were applied to, likely because there was not enough space between the wounds for the tegaderm adhering the dressing to form an adequate seal. We proposed to move the treatment to the A-14-042-TS4 protocol so that we are able to make use of a larger wound diameter, which would result in less wounds per pig but more space in between wounds for the dressing to adhere. We also proposed to use the platform wound device (PWD) to keep the treatments contained to their respective wounds. The PWD has an adhesive rim that creates a strong seal around the wounds, thereby keeping the treatment in place. These modifications to the intramural IACUC was submitted and approved (A-14-042-TP) and was subsequently submitted to ACURO for further review and oversight. The expectation was that upon ACURO approval the studies would be resumed some time in February or March of 2020. However the studies could not be performed since all laboratories were closed due to COVID-19 related shutdowns and stay-at-home orders that started in March 2020.

For both **Aim 2** and **Aim 3** remaining studies, no research could be done as laboratories remained shut until September 2020. This created two issues: (1) the grant award DM160354 was ending in September 2020 and (2) The IACUC periods at both UPitt and ISR were expiring by that time. An additional issue was the anticipated challenge to do pig trauma hemorrhage model studies in the COVD-19 restricted room occupancy framework, because these studies require 5-6 people to be in close quarters within a room. To address these issues, we decided to : (1) Request a no cost extension to our remaining award funds for 12 months (to end in September 2021); (2) Resubmit IACUC and subsequent ACURO approval requests for the animal procedures; and (3) Submit request for a potential model change without changing the scope of the work for the non-rodent trauma hemorrhage model from pigs to rabbits which will still allow evaluating the SP and SP +/- TXA formulations in a DoD-relevant non-rodent trauma model. These three steps were taken accordingly. The 12-month NCE request was granted in Sep 2020. The IACUC and ACURO re-approvals for the pig models were obtained in Dec 2020. The rabbit model explanation and request was sent to the Scientific Officer for the grant and was allowed. Accordingly, IACUC was approved at UPitt for the rabbit trauma hemorrhage model and the resultant documents are currently in preparation for ACURO submission. Upon ACURO submission we anticipate doing the remaining Aim 2 studies in this new model between April-July 2021. In parallel we expect to complete the remaining Aim 3 studies in the pig burn model at Chan Lab (ISR) in the same time. This will allow us to carry out data analysis and report preparation in August and submit to DoD by September of 2021.

Opportunities for Training and Professional Development: During the limited activities of Year 3, the research has allowed the training of two PhD researchers (Aditya Girish and Norman Luc) as well as, several undergraduate researchers (Stephanie Huang, Stephanie Yang, Ankush Banerjee, Yvonne Ma, Kenji Miyazawa, Kelsey Swingle), in various aspects of in vitro, in vivo and ex vivo studies focused on SynthoPlate formulations. These researchers have worked under my mentorship, along with regular consultation with veterinary specialists at the Animal Research Center (ARC) at Case Western, as well as, with our collaborators at University of Pittsburgh and USAISR, to carry out the reported studies. The researchers were also trained in writing technical reports and have contributed heavily towards preparing manuscripts, scientific reports and posters. Similarly, at the University of Pittsburgh, under the guidance of Dr. Matthew Neal, several researchers were trained, including Shannon Halderman, Jurgis Alvikas

and Adnan Hassoune. At ISR, under Dr. Rodney Chan's supervision, Dr. Anders Carlsson is poised to carry out the pig burn model studies with his team.

Results Dissemination: No data was presented in 2020 due to many conference cancellations in the midst of the COVID-19 epidemic.

Plans for next reporting period: 12 month NCE until September 2021
During this period we anticipate to:
- Obtain ACURO approval to carry out remaining Aim 2 studies with SP and SP +/- TXA in a rabbit model of traumatic hemorrhage in Dr. Neal's (Co-I) lab at UPitt.
- Resume pig burn model studies in Dr. Chan's (Co-I) lab at USAISR to evaluate SP and Genta-SP in pig burn wounds.
- Prepare technical report on these activities and prepare appropriate manuscripts and presentations.

4. Impact:

Impact on principal discipline. Not much to report regarding Impact in Year 3, since majority of the studies could not be performed in the midst of COVID-19 related shutdowns, IACUC expirations, grant award expirations, NCE approval, IACUC and ACURO re-approval. While waiting for these issues to resolve, we carried out restricted amount of in vitro studies to see whether we can lyophilize SynthoPlate using Trehalose as a lyo-protectant for potential 'small carry volume' deployable technology for on-field hemorrhage control. These studies yielded promising data regarding the lyophilizability of SP and we plan to study its hemostatic capability in near future. We also continued our extensive literature research on nanoparticle testing in pig models and further confirmed two salient findings from the past reports that, the hemodynamic instability and cardiopulmonary distress are either due to systemic complement activation (CARPA) or organ-specific macrophage activation (pulmonary intravascular macrophages or PIMs) in cloven-hoofed animals. Based upon our studies we do not think that CARPA is an issue in our model, but PIM activation may be a potential issue, and this may not be an issue with humans. Although our studies are in the development phase, our data and design are driven towards a technological solution to reduce hemorrhage-associated preventable deaths of combat personnel in austere battlefield conditions.

Impact on other disciplines. Our studies further strengthened the evidence of 'heteromultivalent surface-decoration' approach for biointeractive nanomedicine. Nanomedicine is a highly significant field of biomedical engineering. These studies expanded the potential of nanomedicine applications in hemorrhage control.

Impact of technology transfer. The studies have added data to our existing patent on SynthoPlate, to expand its use (CIP) in hemorrhage control and field care in trauma.

Impact on society beyond science and technology. The research allowed broader discussions of the potential of military medicine in civilian trauma scenarios with a variety of audience both within and outside the university.

5. Changes/Problems: The biggest challenge of 2020 has been the stalling of research since March 2020 due to COVID-19 related restrictions and laboratory shutdowns. No hands-on in vivo experiments could be performed during March-August 2021. While 'restricted re-opening' happened in September 2021, by this time the grant calendar would have expired and the associated IACUCs and ACURO approvals had also expired. Therefore, we requested and obtained a 12-month no-cost extension (until September 2021) on the grant and have submitted renewal of our IACUCs and obtained (or in the process of obtaining) new ACURO approvals. We hope and anticipate to finish the remaining study components by September 2021.

6. Products: Nothing to report.

7. Participants:

Name:	Anirban Sen Gupta
Project Role:	PI
Researcher Identifier (e.g. ORCID ID):	eRA Commons ID: ANIRBAN0426
Nearest person month worked:	2
Contribution to Project:	Dr. Sen Gupta is the overall director and trainer for the current (and proposed) studies, and mentored all researchers involved.
Funding Support:	NIH R01 HL121212 (PI), NIH R01 HL129179 (PI), NIH R35 GM119526 (Co-I), DM160354 (PI).

Name:	Norman Luc
Project Role:	PhD Graduate Student
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	6
Contribution to Project:	Norman Luc is a graduate researcher in the Sen Gupta laboratory, focusing on evaluating SynthoPlate in vitro and in vivo models. He was responsible for carrying out manufacture and characterization of SynthoPlate for planned studies. He worked with two undergraduate researchers, in a team. He will continue to participate in remaining components of Aim 2 and Aim 3 including manufacture of SP, SP +/- TXA and Genta-SP to ship to collaborator labs for remaining studies.

Funding Support:	NIH R01 HL121212, DM 160354

Name:	Aditya Girish
Project Role:	Masters Student at Case Western
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	6
Contribution to Project:	Aditya Girish is a graduate researcher in the Sen Gupta laboratory, who actively contributes to SynthoPlate™ manufacture, characterization, shipment to collaborator labs, data analysis and report. He is responsible for carrying out the TXA formulation in the clot-targeted particles and its evaluation in vitro using ROTEM. He will continue to participate in remaining components of Aim 2 and Aim 3 including manufacture of SP, SP +/- TXA and Genta-SP to ship to collaborator labs for studies.
Funding Support:	NIH R01 HL 129179, DM160354

Name: Matthew D. Neal, MD
Project Role: co-PI
Research Identifier: Nearest person month worked: 1
Contribution to Project: Dr. Neal leads the experimental design and analysis for all studies proposed under Specific Aim 2. He meets regularly with Dr. Sen Gupta and his team via phone as well as in person, for planning and execution of pig hemorrhage studies as described in Aim 2.

Name: Danielle Reiser
Project Role: animal technician
Research Identifier: Nearest person month worked: 1
Contribution to Project: Ms. Reiser prepared IACUC and ACURO documents for UPitt and coordinated administrative efforts and planning for swine studies.

Name: Shannon Haldeman
Project Role: Research Specialist
Research Identifier: Nearest person month worked: 4
Contribution to Project: Ms. Haldeman is the lead animal surgeon and coordinates all aspects of the swine trauma and hemorrhage model.

Name: Brian S. Zuckerbraun, MD
Project Role: co-I
Research Identifier: Nearest person month worked: 1
Contribution to Project: Dr. Zuckerbraun is an expert in hemorrhagic shock and resuscitation and serves as a consultant for the swine shock models

Name: Jurgis Alvikas, MD
Project Role: General Surgery Research Resident
Research Identifier: Nearest person month worked: 4
Contribution to Project. Dr. Alvikas assists with the animal surgeries and analyzes the data, including blood and organ samples, of the swine trauma and hemmorhage model.

Name: Adnan Hassoune, MD
Project Role: Visiting Research Scholar
Research Identifier: Nearest person month worked: 4
Contribution to Project. Dr. Hassoune assists with the animal surgeries and organizes the data of the swine trauma and hemmorhage model.

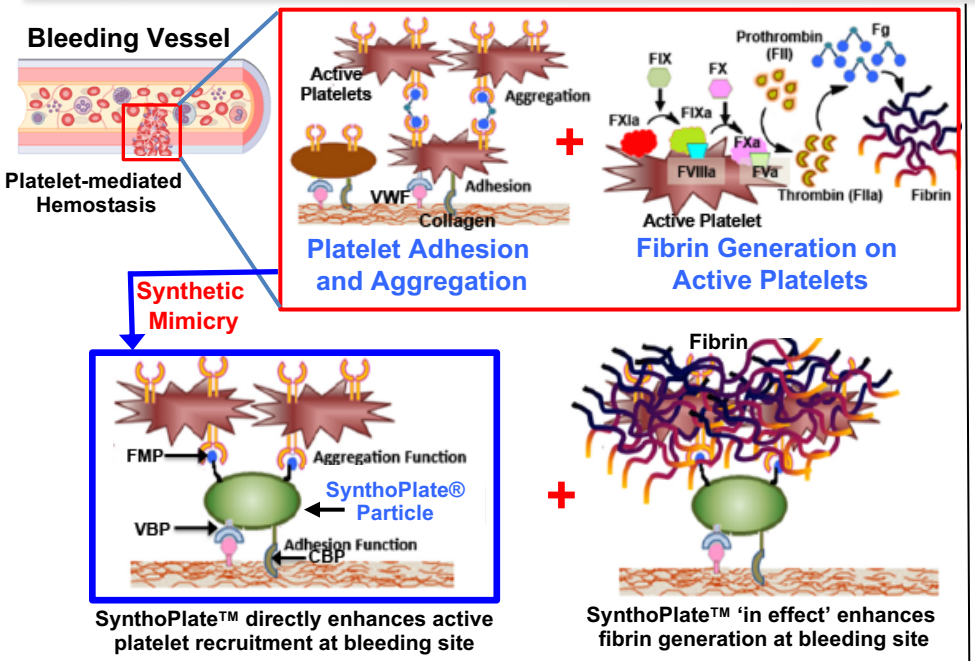
8. Quad Chart: Year 3 Quad Chart attached.

9. Appendices: 12 month NCE approval attached

SynthoPlate™ Nanotechnology For Intravenous Hemostasis and Wound Healing in Prolonged Field Care

Funding Opportunity Number: W81XWH-16-DMRDP-CCCRP-PFCRA

PI: Anirban Sen Gupta, PhD; Co-PI: Matthew D. Neal, MD; Co-I: Ronald Poropatich, MD, Co-I: Rodney K Chan, MD



Military Relevance in Prolonged Field Care

- In PFC and pDCR, transfusion of blood components, especially platelets, can render rapid hemostasis that significantly reduces combat-associated mortalities from trauma and exsanguination
- Platelet products, however, present issues of availability, portability, high bacterial contamination risk, and very short shelf-life that present significant logistical challenges for their efficient point-of-care (pre-MTF, RDCR) use in a far forward military setting
- ***SynthoPlate™*** is a lyophilized powder-form, sterile, shelf-stable, portable ***synthetic platelet technology***, that can be reconstituted in saline at point-of-care, and administered I.V. to mimic, and amplify endogenous mechanisms of platelet-mediated hemostasis
- Beyond rendering efficient hemostasis to reduce morbidity and mortality risks, SynthoPlate™ particles can also act as targeted delivery platforms for spatio-temporally regulated delivery of bioactive agents to promote wound protection and healing

Technology: SynthoPlate™ (synthetic platelet nanotechnology)

Patent US 9107845; Technology Readiness Level: 4

Study Aims (with Milestone Components)

- **Characterize biodistribution, systemic risks and immune response of SynthoPlate™ in pigs**
  - Systemic safety, Maximum Tolerated Dose and Biodistribution
- **Evaluate hemostatic efficacy of SynthoPlate™ alone or in combination with TXA in a swine model of polytrauma**
  - Hemostatic efficacy, Dose response, Dose optimization
  - Effect of SynthoPlate™-TXA combinations on hemostatic efficacy
- **Evaluate efficacy of SynthoPlate™ alone or in combination with Gentamicin for wound protection and healing in pigs**
  - Effect of SynthoPlate™ vs SynthoPlate™-Gentamicin combinations in wound protection and healing in burn and excision wounds

Timeline and Cost

Activities	Year 1	Year 2	Year 3
Systemic safety and biodistribution studies	Complete		
Hemostatic efficacy, dose optimization and TXA-combination studies		In Progress	Stalled due to COVID-19
Wound protection and re-epithelialization studies		In Progress	Stalled due to COVID-19
ESTIMATED BUDGET	$287,734	$357,554	$354,713

TOTAL ESTIMATED BUDGET = $1 Million

AMENDMENT OF SOLICITATION/MODIFICATION OF CONTRACT

1. CONTRACT ID CODE	PAGE OF PAGES
S	1 | 2

2. AMENDMENT/MODIFICATION NO.	3. EFFECTIVE DATE	4. REQUISITION/PURCHASE REQ. NO.	5. PROJECT NO.(If applicable)
P00001	30-Sep-2020	0011004310-0001	

6. ISSUED BY	CODE	7. ADMINISTERED BY (If other than item 6)	CODE
USA MED RESEARCH ACQ ACTIVITY 820 CHANDLER ST FORT DETRICK MD 21702-5014	W81XWH	See Item 6	

8. NAME AND ADDRESS OF CONTRACTOR (No., Street, County, State and Zip Code)		
CASE WESTERN RESERVE UNIVERSITY 10900 EUCLID AVE CLEVELAND OH 44106-1712		9A. AMENDMENT OF SOLICITATION NO.
		9B. DATED (SEE ITEM 11)
	X	10A. MOD. OF CONTRACT/ORDER NO. W81XWH-17-2-0064
	X	10B. DATED (SEE ITEM 13) 30-Sep-2017
CODE 4B566	FACILITY CODE	

11. THIS ITEM ONLY APPLIES TO AMENDMENTS OF SOLICITATIONS

☐ The above numbered solicitation is amended as set forth in Item 14. The hour and date specified for receipt of Offer ☐ is extended, ☐ is not extended.

Offer must acknowledge receipt of this amendment prior to the hour and date specified in the solicitation or as amended by one of the following methods: (a) By completing Items 8 and 15, and returning _____ copies of the amendment; (b) By acknowledging receipt of this amendment on each copy of the offer submitted; or (c) By separate letter or telegram which includes a reference to the solicitation and amendment numbers. FAILURE OF YOUR ACKNOWLEDGMENT TO BE RECEIVED AT THE PLACE DESIGNATED FOR THE RECEIPT OF OFFERS PRIOR TO THE HOUR AND DATE SPECIFIED MAY RESULT IN REJECTION OF YOUR OFFER. If by virtue of this amendment you desire to change an offer already submitted, such change may be made by telegram or letter, provided each telegram or letter makes reference to the solicitation and this amendment, and is received prior to the opening hour and date specified.

12. ACCOUNTING AND APPROPRIATION DATA (If required)

13. THIS ITEM APPLIES ONLY TO MODIFICATIONS OF CONTRACTS/ORDERS. IT MODIFIES THE CONTRACT/ORDER NO. AS DESCRIBED IN ITEM 14.

☐ A. THIS CHANGE ORDER IS ISSUED PURSUANT TO: (Specify authority) THE CHANGES SET FORTH IN ITEM 14 ARE MADE IN THE CONTRACT ORDER NO. IN ITEM 10A.

☐ B. THE ABOVE NUMBERED CONTRACT/ORDER IS MODIFIED TO REFLECT THE ADMINISTRATIVE CHANGES (such as changes in paying office, appropriation date, etc.) SET FORTH IN ITEM 14, PURSUANT TO THE AUTHORITY OF FAR 43.103(B).

☐ C. THIS SUPPLEMENTAL AGREEMENT IS ENTERED INTO PURSUANT TO AUTHORITY OF:

X D. OTHER (Specify type of modification and authority)
IAW USAMRAA Terms & Conditions

E. IMPORTANT: Contractor ☒ is not, ☐ is required to sign this document and return _____ copies to the issuing office.

14. DESCRIPTION OF AMENDMENT/MODIFICATION (Organized by UCF section headings, including solicitation/contract subject matter where feasible.)
Modification Control Number: rdoan204760

PROJECT TITLE: SynthoPlate Nanotechnology for Intravenous Hemostasis and Wound Healing in Prolonged Field Care
PRINCIPAL INVESTIGATOR: Anirban Sen Gupta
PERIOD OF PERFORMANCE: 30 September 2017 - 29 September 2021
TOTAL AWARD AND FUNDED AMOUNT: $969,214.00

The purpose of this modification is to extend the period of performance by 12 months, at no cost to the Government, per the recipient's request. All reporting requirements shall continue during this extension period. The next annual technical report is due 30 October 2020. The final technical report is due 30 January 2022. See Summary of Changes.

Except as provided herein, all terms and conditions of the document referenced in Item 9A or 10A, as heretofore changed, remains unchanged and in full force and effect.

15A. NAME AND TITLE OF SIGNER (Type or print)		16A. NAME AND TITLE OF CONTRACTING OFFICER (Type or print) ELENA G. HOWELL / GRANTS OFFICER TEL: 301-619-6871 EMAIL: elena.g.howell.civ@mail.mil	
15B. CONTRACTOR/OFFEROR (Signature of person authorized to sign)	15C. DATE SIGNED	16B. UNITED STATES OF AMERICA BY Elena A Howell (Signature of Contracting Officer)	16C. DATE SIGNED 10-Sep-2020

SECTION SF 30 BLOCK 14 CONTINUATION PAGE

SUMMARY OF CHANGES

SECTION 00010 - SOLICITATION CONTRACT FORM

DELIVERIES AND PERFORMANCE

The following Delivery Schedule item for CLIN 0001 has been changed from:

DELIVERY DATE	QUANTITY	SHIP TO ADDRESS	DODAAC / CAGE
POP 30-SEP-2017 TO 29-SEP-2020	N/A	W03J USA MED RESEARCH MAT CMD W03J USA MED RESEARCH MAT CMD 1077 PATCHEL STREET FORT DETRICK MD 21702-5024 301-619-7416 FOB: Destination	W91ZSQ

To:

DELIVERY DATE	QUANTITY	SHIP TO ADDRESS	DODAAC / CAGE
POP 30-SEP-2017 TO 29-SEP-2021	N/A	W03J USA MED RESEARCH MAT CMD W03J USA MED RESEARCH MAT CMD 1077 PATCHEL STREET FORT DETRICK MD 21702-5024 301-619-7416 FOB: Destination	W91ZSQ

(End of Summary of Changes)