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PRINCIPAL INVESTIGATOR: Anirban Sen Gupta

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Anirban Sen Gupta
email: anirban.sengupta@case.edu

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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.

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None listed.

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TABLE OF CONTENTS

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 Ioban 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 were 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) at 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.

Year 4 (Months 36-48)

As described in Quarterly Reports and Final Report of Year 3, we requested a 'change of model' for Sp Aim 2 from swine to rabbit, and this was approved. Hence the **revised Sp Aim 2** is:

Sp Aim 2: Evaluate hemostatic efficacy of SynthoPlate doses in Rabbit Model of uncontrolled hemorrhage

Major Task 1: Evaluate post-injury SynthoPlate transfusion for reducing blood loss following trauma injury

Subtask 1: *IACUC and ACURO approval for Rabbit Model*

Subtask 2: *SynthoPlate and Control particle manufacture and supply to UPitt for study in rabbit model*

Subtask 3: *Studies with Sham animals and animals injected with control (unmodified) particle*

studies: rabbit model injected with sham or control particles and evaluation of hemorrhage outcome, and analysis serum cytokines for determination of systemic inflammation levels (baseline)
Subtask 4: *Studies on animals injected with SynthoPlate doses and evaluation of hemostatic effect, survival and analysis serum cytokines for assessment of systemic inflammation and systemic thrombotic side effects (if any).*

Regarding Subtask 1 under Major Task 1 of Revised Specific Aim 2, the Rabbit model IACUC was approved at UPitt (Neal Lab) in December, 2020. Accordingly, ACURO documents were prepared in January 2021 and submitted to DoD in February 2021. ACURO review of the UPitt IACUC led to a several questions and commentary for clarifications, and the IACUC documents were sent back to UPitt for review and amendment. In review of the IACUC, several amendments and corrections were required, which were carried out and re-submitted for IACUC approval. We are awaiting the amended IACUC approval, to then submit back to ACURO for approval. Due to anticipated lag-time regarding this amended IACUC approval at UPitt and subsequent review and approval by DoD ACURO, we communicated with the Scientific Officer and decided to submit a request for second NCE to be able to complete the Major Task 1 studies during Fall 2021 and Spring 2022. This second NCE request was submitted in September 2021 and was approved. Therefore, the Major Task 1 studies were anticipated to be done during Fall 2021-Spring 2022. The amended IACUC was also received from UPitt and incorporated into new ACURO evaluation document to carry out these studies.

Major Task 2: Major Task 2: Identify the effects of SynthoPlate ± TXA blood loss and hemodynamic changes following traumatic injury.

This specific major task component was canceled due to budgetary limitation. The limitation stemmed from the fact that during 2020 although no experimental work could be carried out regarding Aim 2 due to COVID-related lab shutdown, the research personnel salary components still needed to be covered. This resulted in a part reduction of 'residual budget' for Aim 2 in 2021, which necessitated partial reduction of proposed Aim tasks. Since the primary focus of the project is the evaluation of SynthoPlate, and the context of 'SynthoPlate + TXA' evaluation is secondary to it, we decided to cancel this secondary task to be able to cover the primary task with the residual budget. This aspect was communicated with the Scientific Officer.

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

IACUC and ACURO approval for these studies were achieved during Spring/Summer 2021. Gentamicin-loaded SP was shipped to ISR in March 2021 and the swine burn model studies were resumed. The studies were carried for 90-day time span. Deep partial-thickness burns were induced by a thermocoupled 5cm diameter, brass burn device at 100°C for a duration of 15 seconds with a constant application force. The wounds were lightly debrided by cleaning with a

wet cotton gauze and topical treatments were applied in a special device using a polyurethane device – Platform wound device (PWD). Assessments and reapplications of treatments occur on days 3,7, 14, 21 and 28. The wounds are then followed with assessments and biopsies on days 60 and 90 which is the final endpoint. The wounds are likely re-epithelialized at day 28, so there were not any further treatment applications at that point. Scarring and epithelialization outcomes were assessed on a monthly schedule until day 90. At days 28, 60 and 90 biopsies were harvested for histology. In addition, on day 90 a large excisional strip biopsy was harvested postmortem for histological scar assessments. The overall study results showed slightly higher re-epithelialization for SynthoPlate-treated group but no statistically significant differences between the various treatment groups. Wounds treated with SynthoPlate were ~96% re-epithelialized whereas those treated with the standard-of-care (SOC, Silver sulfadiazine ointment) measured slightly less at ~92% (p= 0.56). Re-epithelialization in the Vehicle Control (Saline) group was ~97% and in the Gentamicin group was ~90%. In conclusion, re-epithelialization in wounds treated by SynthoPlate were slightly improved over the SOC, but this difference did not reach significance. Interestingly, when SynthoPlate was used in combination with Gentamicin, there was a trend showing that the mean re-epithelialization was decreased relative to both the SOC and Gentamicin alone. The percentage of wound contraction was monitored by applying a tattoo to the borders of the area intended for burn creation on day 0. This tattooed area was then measured on each day of analysis and recorded. The mean area for each treatment group was calculated at day 90. The percentage of wound contraction was reported as a ratio of the size of the wound after 90 days of healing with its respective treatment relative to the initial wound size. A growth co-efficient was factored in, to account for porcine development and growth over the 90-day period in order to scale size changes not due to contraction. The results showed that no statistically significant differences were observed between the treatment groups. The wound contraction in the SOC group was the lowest with wounds measuring 93% of their initial size (Figure 2). Wound contraction in wounds treated with SynthoPlate was higher with wounds measuring 86% of their initial size. Relative to Synthoplate alone, contraction improved in wounds treated in combination with Gentamicin at the wounds measured 91% of their initial size (p= 0.67). In the Vehicle Control (Saline) group, the wounds measured at 85% of their initial size and in the Gentamicin alone group the wound contraction percentage was 91% of initial wound size. In conclusion, the percentage of wound contraction in the treatment groups of SynthoPlate as well as SynthoPlate with Gentamicin did not improve outcomes relative to the SOC. Although mean percentage of wound contraction was improved in the combination treatment group of SynthoPlate with Gentamicin relative to SynthoPlate alone, this difference did not reach significance. Superficial blood flow was monitored through Laser Speckle analysis. Data was recorded at each assessment and wounds were monitored for their amount of superficial blood flow relative to normal, uninjured control skin to compare levels of blood flow. This analysis served as a surrogate for angiogenesis. At post-burn day 28, in the SynthoPlate treatment group, superficial blood flow was measured to be 168% of normal skin controls. Meanwhile in wounds treated with a combination of SPM with gentamicin, superficial blood flow was slightly lower at 160%. In wounds treated with the SOC, superficial blood flow decreased to 110% of normal skin controls (p= 0.27). In conclusion, superficial blood was elevated well above that of normal skin controls in the SynthoPlate treatment group as well as the SynthoPlate with Gentamicin group at post-burn day 28. These treatments were improved also in regard to the treatment with the SOC, but this observed difference did not reach significance. Wounds were monitored for the amount of bacterial load present in order to assess how well protected from infection each treatment group would be as they progressed through healing. Bacterial swabs were collected and cultured. The mean bacterial load in each group was assessed at post-burn day 3 through visual assessment with the MolecuLight Wound Imaging Device that allows visualization of bacteria on the culture dish. A scoring system was developed to stratify the amount of bacteria present in accordance with visual representation from 0-100 where higher the score greater the burden of bacteria.

Notably, this consisted of the mean bacterial load being the lowest in wounds treated with gentamicin alone at 17/100. Meanwhile in wounds treated with the SOC, or for those in the SynthoPlate with gentamicin group, mean bacterial loads were significantly higher at 43/100 and 47/100, respectively ($p = 0.02$). Mean bacterial load of SynthoPlate alone was 53/100 and for wounds in the Vehicle Control (Saline) group was 57/100. In conclusion, Gentamicin decreased the amount of bacteria present on the wounds significantly. SynthoPlate did not decrease bacterial load relative to the SOC. However, when in combination with Gentamicin, the mean bacterial load was decreased although this did not reach significance. The vehicle control (saline) group had the highest bacterial burden. These values served as representations of the antibacterial properties offered by each type of treatment to deep partial-thickness burns. The overall conclusion altogether from these studies is that SynthoPlate alone as well as Gentamicin-loaded SynthoPlate show a trend towards improved epithelilization and blood flow (characteristics of improved healing), but at the current stage this observed trend has not reached a statistical significance. We envision that future studies, supported by subsequent contract awards from DoD will enable us to improve upon this by refining the SynthoPlate design to make it capable of augmenting fibrin and collagen formation (pro-healing matrix proteins), as well as delivering other therapeutic agents beyond Gentamicin.

Opportunities for Training and Professional Development: During the limited but resumed activities of Year 4 in the midst of COVID vaccinations and somewhat restricted lab operations, 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 contribute to the reported studies. The researchers were also trained in writing technical reports. Similarly, at the University of Pittsburgh, under the guidance of Dr. Matthew Neal, several researchers were trained, including Shannon Halderman, Jurgis Alvikas, Adnan Hassoune and Roberto Mota. At ISR, under Dr. Rodney Chan's and Dr. Anders Carlsson's supervision, the team completed remaining Aim 3 studies in the pig burn model.

Results Dissemination: Aim 3 results were submitted as a research presentation abstract to the American Burn Association 2022 annual meeting. Please note that research data could not be presented at MHSRS 2021 as the meeting was canceled due to continued COVID-19 obstacles.

Plans for next reporting period: 12 month NCE until September 2022
During this period we anticipate to:
- Obtain ACURO approval to carry out remaining Aim 2 studies with SP in a rabbit model of traumatic hemorrhage in Dr. Neal's (Co-I) lab at UPitt.
- Prepare research presentation and technical abstracts for conference presentations and manuscripts stemming from the studies.

4. Impact:

Impact on principal discipline. In Year 4 (2021 which was NCE Year 1), remaining Aim 3 studies were completed, generating the first volume of in vivo preliminary data on SynthoPlate effect in burn wound healing. In parallel to this, funded by a separate DoD contract (PRMRP, PR 191632), 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 have yielded very promising data regarding the

lyophilizability of SP and these findings are complementary to the studies being carried out with DM 160354 funds, to potentially lead to an on-field applicable hemorrhage control and wound care technology. Although our studies are in the research and development phase, our data and design focused on a technological solution to reduce hemorrhage-associated preventable deaths of combat personnel in austere battlefield conditions, has high translational promise.

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 2021 has been the sporadic stalling of research activities, as well 'limited occupancy in labs' scenario, due to continued issues associated with COVID-19 obstacles. Nonetheless, we were able to complete our remaining studies for Aim 3. The Aim 2 rabbit studies with SynthoPlate had to be postponed due to delays in IACUC amendments and ACURO approval, and thus a second NCE has been requested (now approved) to carry out this part of the studies during Spring 2022. Barring any major surge of COVID problems (e.g. the Omicron variant related issues) we hope and anticipate to finish the remaining study components by June/July 2022, and submit final report by September 2022.

6. Products: Aim 3 results were submitted as a research presentation abstract to the American Burn Association 2022 annual meeting..

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 R01 HL141080 (MPI), DM160354 (PI), PR 191632 (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 trauma and hemorrhage models.

Name: Adnan Hassoune, MD
Project Role: Visiting Research Scholar
Research Identifier: Nearest person month worked: 4Contribution to Project. Dr. Hassoune assists with the animal surgeries and organizes the data for studies on trauma and hemorrhage models.

Name: Rodney Chan, MD
Project Role: Subaward PI
Research Identifier: https://orcid.org/0000-0002-5061-847X
Nearest person month worked: 1.80 calendar months
Contribution to Project: Data Analysis, Animal Experiments

Name: Anders Carlsson, PhD
Project Role: Subaward Co-PI
Research Identifier: https://orcid.org/0000-0002-4846-108X
Nearest person month worked: 6.00 calendar months
Contribution to Project: Data Analysis, Animal Experiments

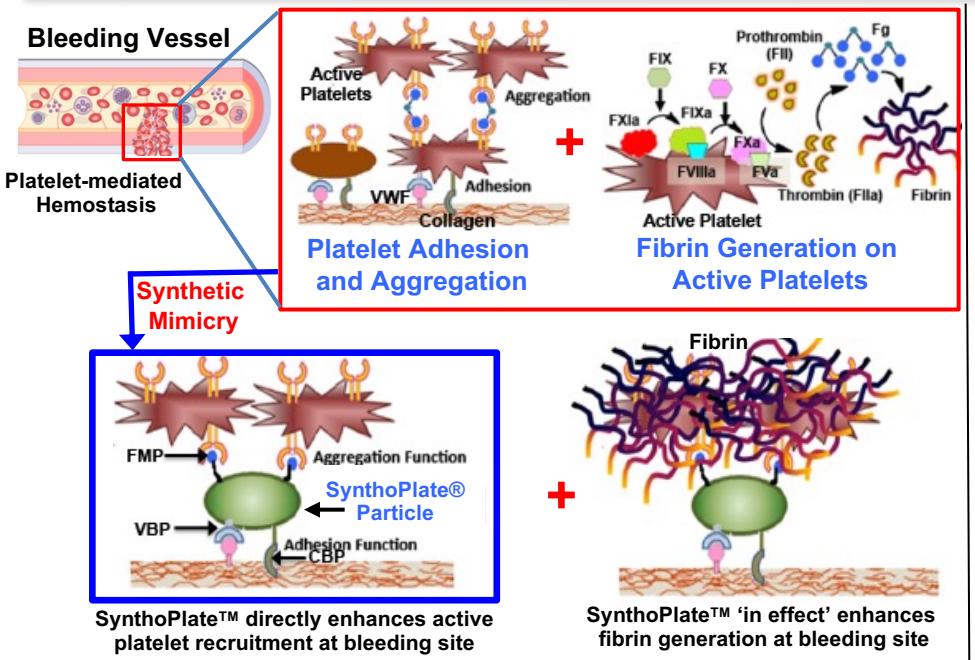
8. Quad Chart: Year 4 Quad Chart attached.

9. Appendices: 12 month second 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)

- **Aim 1: Characterize biodistribution, systemic risks and immune response of SynthoPlate™ in pigs**
  - Systemic safety, Maximum Tolerated Dose and Biodistribution
- **Revised Aim 2: Evaluate hemostatic efficacy of SynthoPlate™ in a rabbit model of trauma hemorrhage**
  - Hemostatic efficacy, Dose response, Dose optimization
- **Aim 3: 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 & 4
Systemic safety and biodistribution studies	Complete		
Hemostatic efficacy studies in vivo (pig model originally, revised rabbit model of trauma in Year 3)		In Progress (second NCE received)	
Wound protection and re-epithelialization studies		Complete	
ESTIMATED BUDGET	$287,734	$357,554	$354,713

TOTAL ESTIMATED BUDGET = $1 Million

STATEMENT OF WORK – Month/Day/Year
PROPOSED START DATE October 1, 2017

Site 1: Case Western Reserve Univ
Dept of Biomedical Engineering,
10900 Euclid Avenue
Wickenden Bldg Rm 517B
Cleveland, OH 44106
PI: Dr. Anirban Sen Gupta

Site 2: Univ of Pittsburgh
Dept of Surgery, F1271.2 PUH
200 Lothrop Street
Pittsburgh, PA 15213
PI: Dr. Matthew D Neal

Site 3: United States Army Institute for Surgical Research (USAISR)
3968 Chambers Pass STE B, JBSA Fort Sam Houston
San Antonio Military Medical Center, Bldg 3611
PI: Rodney K, Chan, MD, FACS, FRCSC (RKC)

ANNOTATED FOR SECOND NCE PERIOD: Requested Second NCE October 1, 2021-September 30, 2022 (12 months)

- Already completed tasks and milestones (2017-2019)
- Tasks to be completed by Sep 2021
- Tasks and milestones to be completed during second NCE period (Oct 1, 2021-Sep 30, 2022)
- Tasks canceled as they cannot be achieved with remaining funds

Specific Aim 1 (Characterize biodistribution, systemic risks and immune response of SynthoPlate™ in pigs)	**Timeline: Mo 1-12**	**Site 1**	**Site 2**	**Site 3**
Major Task 1: *Characterize and mitigate immune response (if any) to SynthoPlate dosing*	Mo 1-9	X		
Subtask 1: SynthoPlate manufacture and sterilization (platelet-mimetic nanoparticle manufacture from lipid-peptide conjugates using reverse phase evaporation and extrusion; sterilization with E-beam)	Mo 1,3,6,9	Dr. Sen Gupta		
Subtask 2: IACUC and ACURO approval, site: CWRU	Mo 1-3	Dr. Sen Gupta		
Subtask 3: Complement and IgG analysis on various doses (UV-Visible Spectroscopy based biochemical assays for complement activation analysis for C3a and C5a in plasma, upon administration of SynthoPlate™ doses)	Mo 3-9	Dr. Sen Gupta (25 pigs)		
Major Task 2: *Characterize and mitigate systemic pro-thrombotic risks (if any) upon SynthoPlate administration*	Mo 1-9	X		
Subtask: Monitoring blood pressure, heart rate, coagulation parameters, platelet aggregometry, ROTEM analysis, D-dimer, on blood samples drawn from and excised organ histology of clearance organs (lungs, heart, kidney, liver, spleen) for the	Mo 3-9	Dr. Sen Gupta (same 25 pigs as above)		

various doses of SynthoPlate used in the pigs in Subtask 3 above				
Major Task 3: *Characterize biodistribution of SynthoPlate over time*	Mo 1-9	X		
Subtask: Rhodamine-B fluorescence-based liquid chromatographic analysis of SynthoPlate sequestration and distribution in excised organs from animals during 1 hr period	Mo 3-9	Dr. Sen Gupta (same 25 pigs as above)		
Major Task 4: *Establish a safe dosing protocol for SynthoPlate™ in pigs*	Mo 9-12	X		
Subtask: Statistical analysis of all data generated during Mo 3-9, generation of progress report and safe dosage protocol documents to supply to collaborators	Mo 9-12	Dr. Sen Gupta		
Milestones: 1. Characterization and mitigation of immune response (if any) to SynthoPlate dosing 2. Characterization of biodistribution of SynthoPlate over time 3. Characterization and mitigation of systemic pro-thrombotic risks (if any) upon SynthoPlate administration, 4. Establish a safe dosing protocol for SynthoPlate™ in pigs.	Mo 3-12	Dr. Sen Gupta		

Specific Aim 2 **(Evaluate hemostatic efficacy of pristine SynthoPlate™ and TXA-loaded SynthoPlate™ in porcine model of polytrauma.)**	**Timeline: Mo 6-36**	**Site 1**	**Site 2**	**Site 3**
Major Task 1: *Demonstrate that post-injury SynthoPlate transfusion results in a significant reduction in blood loss following polytrauma*	Mo 6-24	X	X	
Subtask 1: SynthoPlate manufacture, sterilization, supply to UPitt: (platelet-mimetic nanoparticle manufacture from lipid-peptide conjugates using reverse phase evaporation and extrusion; sterilization with E-beam, shipment	Mo 6,9, 12, 15, 18	Dr. Sen Gupta		

of SynthoPlate vials to UPitt)				
Subtask 2: IACUC and ACURO approval, UPitt	Mo 6-9		Dr. Neal	
Subtask 3: Studies with Sham animals and animals injected with control (unmodified) particle studies	Mo 10-15		Dr. Neal (10 animals total)	
Subtask 4: Studies on polytrauma animals (pigs with polytrauma injury and hemorrhage) injected with SynthoPlate doses and evaluation of hemostatic effect and analysis serum cytokines for mitigation benefit of systemic inflammation	Mo 15-22		Dr. Neal (7 animals)	
Subtask 5: Statistical analysis, report generation, manuscript preparation on Major Task 1 of Sp Aim 2	Mo 20-24	Dr. Sen Gupta	Dr. Neal	
Revised Specific Aim 2 (NCE Period, Months 48-56) Evaluate hemostatic efficacy of SynthoPlate doses in **Rabbit Model** of uncontrolled hemorrhage	Mo 48-60	Dr. Sen Gupta	Dr. Neal	
Major Task 1: *Evaluate post-injury SynthoPlate transfusion for reducing blood loss following trauma injury*	Mo 48-52	Dr. Sen Gupta	Dr. Neal	
Subtask 1: IACUC and ACURO approval for Rabbit Model	Mo 48-52		Dr. Neal	
Subtask 2: SynthoPlate and Control particle manufacture and supply to UPitt for study in rabbit model	Mo 48-54	Dr. Sen Gupta	Dr. Neal	
Subtask 3: Studies with Sham animals and animals injected with control (unmodified) particle studies: rabbit model injected with sham or control particles and evaluation of hemorrhage outcome, and analysis serum cytokines for determination of systemic inflammation levels (baseline)	Mo 50-54		Dr. Neal (5 sham + 5 control = 10 animals)	
Subtask 4: Studies on animals injected with SynthoPlate doses and evaluation of hemostatic effect, survival and analysis serum cytokines for assessment of systemic inflammation and systemic thrombotic side effects (if any).	Mo 52-58		Dr. Neal (10 animals)	

Subtask 5: Statistical analysis, report generation, abstract and manuscript preparation on Sp Aim 2 studies	Mo 58-60			
Major Task 2: *Identify the effects of SynthoPlate ± TXA blood loss and hemodynamic changes following traumatic injury.*		CANCELED	CANCELED	
Subtask 1: Manufacture and characterization of SynthoPlate loaded with TXA (TXA loading and release kinetics characterization from SynthoPlate particles) and shipment of TXA-loaded nanoparticles and control nanoparticles to UPitt				
Subtask 2: Studies on rabbit hemorrhage model injected with control particle + TXA vs SynthoPlate + TXA for hemostatic efficacy (hemorrhage control)				
Major Task 3: Data analysis, Report generation, manuscript preparation, next steps of milestones and funding strategy discussions				
Subtask : Serum measurements of cytokines, histology studies on excised organ sections for evaluating organ protection and anti-inflammation benefits				
Major Task 4: *Final Report Generation on Sp Aim 2*				
Milestones: 1. Demonstrate that post-injury SynthoPlate transfusion results in a significant reduction in blood loss following hemorrhagic trauma (hemostatic efficacy). 2: Evaluate the potential synergistic effect of SynthoPlate and site-specific delivery of TXA on blood loss and hemodynamic changes after injury (hemostatic efficacy).				

3. Identify the effects of SynthoPlates ± TXA on organ injury protection and inflammation following trauma. 4. Submit manuscript to peer-reviewed journal for publication				

Specific 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.)	**Timeline: Mo 6-36**	**Site 1**	**Site 2**	**Site 3**
Major Task 1: *Demonstrate that SynthoPlate™ alone has beneficial effect on wound protection and inflammation*	Mo 6-18	X		X
Subtask 1: CRADA establishment with Dr. Chan's lab, USAISR	Mo 6-8	Dr. Sen Gupta		Dr. Chan
Subtask 1: SynthoPlate manufacture, sterilization, ship to USAISR (Dr. Chan) by Mo 12	Mo 6,9, 12	Dr. Sen Gupta		
Subtask 2: Studies with localized application of SynthoPlate on burn wound in pigs for healing efficacy	Mo 12-15			Dr. Chan
Subtask 3: Report development on Major Task 1	Mo 15-18	Dr. Sen Gupta		Dr. Chan
Major Task 2 *Demonstrate that SynthoPlate™-Gentamicin combinations provide wound protection and decrease inflammation by decreasing the bacterial burden and local inflammatory markers*	**Mo 36-48 (NCE Period)**	X		
Subtask 1: Manufacture and characterization of SynthoPlate + Gentamicin formulations (loading + release), supply to Dr. Chan	Mo 36-44	Dr. Sen Gupta		
Subtask 2: Studies with SynthoPlate + Gentamicin in pig burn wounds	Mo 36-44			Dr. Chan (2 pigs)
Subtask 3: Data analysis, Final Report generation on Major Task 2, manuscript preparation	Mo 44-46	Dr. Sen Gupta		Dr. Chan
Major Task 3: *Demonstrate that*	**Mo 44-48**	X		X

SynthoPlate™ alone, or with Gentamicin combination (additive or encapsulated within), has a beneficial effect on re-epithelialization of a wound				
Subtask 1: Manufacture SynthoPlate + Gentamicin formulations to supply to Dr. Chan, as before	Mo 44-47	Dr. Sen Gupta		
Subtask 2: Studies of SynthoPlate + Gentamicin in pig excision wounds to evaluate healing response (epithelialization)	Mo 44-47			Dr. Chan (same 2 pigs)
Major Task 4: *Final Report Generation on Sp Aim 3*	Mo 47, 48	Dr. Sen Gupta		Dr. Chan
Subtask: Report generation, manuscript preparation, next steps research/funding strategy discussion	Mo 47, 48	Dr. Sen Gupta		Dr. Chan
Milestones: 1: Demonstrate that SynthoPlate™ alone has beneficial effect on wound protection and inflammation 2:Demonstrate that SynthoPlate™-Gentamicin combinations provide wound protection and decrease inflammation by decreasing the bacterial burden and local inflammatory markers, respectively 3: Demonstrate that SynthoPlate™ alone, or with Gentamicin combination (additive or encapsulated within), has a beneficial effect on re-epithelialization of a wound. 4. Submit manuscript to peer-reviewed journal for publication		Dr. Sen Gupta		Dr. Chan

AMENDMENT OF SOLICITATION/MODIFICATION OF CONTRACT

1. CONTRACT ID CODE	PAGE OF PAGES
S	1 | 16

2. AMENDMENT/MODIFICATION NO.	3. EFFECTIVE DATE	4. REQUISITION/PURCHASE REQ. NO.	5. PROJECT NO.(If applicable)
P00002	30-Sep-2021	0011004310-0001	

6. ISSUED BY	CODE	7. ADMINISTERED BY (If other than item 6)	CODE
US ARMY MEDICAL RESEARCH ACQUISITION ACT DIRECTOR 820 CHANDLER STREET 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 Award T/Cs and Mutual Agreement

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: rdoan215326

PROJECT TITLE: SynthoPlate Nanotechnology for Intravenous Hemostasis and Wound Healing in Prolonged Field Care
PRINCIPAL INVESTIGATOR: Dr. Anirban Sen Gupta
PERIOD OF PERFORMANCE: 30 September 2017 - 29 September 2022
TOTAL AWARD AND FUNDED AMOUNT:
The purpose of this modification is to exten the period of performance by 12 months, at no additional cost to the Government, per the recipient's request 21 September 2021. An annual technical progress report is due 30 October 2021. The final technical report is due within 120 days of the POP expiration. Additionally, the revised Statement of Work (SOW) dated 21 September 2021 is incorporated herein by reference. See the Summary of Changes on the following pages.
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) JOSHUA MCKEAN / GRANTS OFFICER TEL: 301-619-4046 EMAIL: joshua.d.mckean3.civ@mail.mil	
15B. CONTRACTOR/OFFEROR _____ (Signature of person authorized to sign)	15C. DATE SIGNED	16B. UNITED STATES OF AMERICA BY _____ (Signature of Contracting Officer)	16C. DATE SIGNED 29-Sep-2021

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-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

To:

DELIVERY DATE	QUANTITY	SHIP TO ADDRESS	DODAAC / CAGE
POP 30-SEP-2017 TO 29-SEP-2022	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

SECTION 00800 - SPECIAL CONTRACT REQUIREMENTS

The following have been modified:

U.S. ARMY MEDICAL RESEARCH ACQUISITION ACTIVITY
AWARD SPECIFIC RESEARCH TERMS AND CONDITIONS
WITH INSTITUTIONS OF HIGHER EDUCATION, HOSPITALS, AND NON-PROFIT ORGANIZATIONS

DIVISION I – AWARD COVER PAGES

Principal Investigator Dr. Anirban Sen Gupta

Project Title SynthoPlate Nanotechnology for Intravenous Hemostasis and Wound Healing in Prolonged Field Care

Technical Abstract

A. Background. 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 variability's. 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.

B. Objective. We seek to evaluate the point-of-care hemostatic efficacy and spatio-temporally targeted wound healing treatment applicability of the SynthoPlateTM nanotechnology in appropriate porcine models, with a vision to translate this technology for prolonged combat casualty care in a far forward setting.

C. Specific Aims. We propose to meet our objective under the following specific aims:

- Aim 1. Characterization of biodistribution, systemic risks and immune response of intravenously administered SynthoPlateTM in pigs.
- Aim 2. Evaluation of hemostatic efficacy of pristine SynthoPlateTM and TXA-loaded SynthoPlateTM in a pig model of polytrauma.
- Aim 3. Evaluate the efficacy of SynthoPlateTM alone or in combination with Gentamicin to provide wound protection and improve re-epithelialization in porcine wound models.

D. Study Design. For Aim 1, we will administer fluorescently-labeled SynthoPlateTM at selected doses in 50 ml suspension volume via jugular vein in pigs, and monitor vitals over 1 hr, during which periodic blood draws will be performed to evaluate CBC, complement activation levels, and systemic pro-thrombotic and pro-coagulant risks via platelet aggregometry, D-dimer assay and thromboelastometry. Based upon initial findings, repeat dosing will be performed after 15 and 30 min. Post 1 hr, animals will be euthanized and harvested organ homogenates will be analyzed chromatographically for SynthoPlateTM sequestration (fluorescence-based biodistribution) and histopathologically to assess organ-specific microvascular thrombi risks. For Aim 2, the safe dosage protocol established via Aim 1 will be used to administer empty (pristine) SynthoPlateTM, or SynthoPlateTM added to TXA, or SynthoPlateTM loaded inside with TXA, to pig model of polytrauma (femur crush + liver injury + lung contusion) and hemorrhagic shock, to evaluate comparative hemostatic efficacy (monitoring blood loss and hemodynamics) over 90 min period. Treatment with just saline will be used as control. Post-euthanasia, tissues/organs will be harvested to analyze SynthoPlate sequestrationTM and organ injury. For Aim 3, first the ability of SynthoPlateTM itself, or SynthoPlateTM + Gentamicin combinations (additively mixed or loaded within vesicle) to provide wound protection (anti-infection effect) and reduce inflammation will be investigated in a deep partial-thickness burn model in pigs. On post-burn day 7, pigs will be euthanized, the superficial skin harvested and analyzed for bacterial burden and inflammatory cytokines. Based upon the findings the SynthoPlateTM or SynthoPlateTM-Gentamicin combinations will be further investigated for their ability to improve re-epithelialization in tangential excision model in pigs observed over 28 days (wound assessed on days 7, 14, 21 and 28). Standard of care or no treatment will be used as controls.

E. Impact. Our proposed research can impact several focus areas in the PFCRA program. In Focus Area 1, the SynthoPlateTM technology can become an alternative or adjunctive hemostatic modality for prolonged (over 2-4 hours) field care in pre-hospital military settings. In Focus Area 2, the technology can become an advanced intravenous hemostatic approach to decrease morbidity and mortality in out-of-hospital resuscitation and wound stabilization for the military, including in TBI. Establishing its targeted hemostatic and wound healing potential in the military via our research can further lead to its civilian use for emergency hemostatic management.

A. Objective and Rationale for the Research. Combat injury-related uncontrolled non-compressible bleeding can lead to multiple organ damage and this remains a leading cause of death for military wounded in combat. A clinical standard for treating such scenarios is transfusion of whole blood or blood products, especially platelets which are

small cell fragments in the blood that facilitate blood clotting at the injury site and reduce the risks of mortality. Platelets also secrete a cocktail of biological molecules that facilitate tissue repair and wound healing over time, which has made local application of platelet-rich plasma gel an effectively modality in wounded tissue treatment. However, accessibility to platelet transfusions and other platelet products is a tremendous logistical challenge in the remote austere conditions of the battlefield, due to their: (1) Limited availability via blood type matching; (2) Limited portability and special storage requirements to preserve biological/clotting activity; (3) High risk of bacterial contamination; (4) Very short shelf-life (3-5 days); (5) Batch-to-batch variability in clotting capabilities. We rationalize that these issues can be potentially resolved by creating synthetic particles that (a) mimic platelet's mechanism of blood clotting, (b) will have minimum immunogenicity risk if made from biocompatible and biodegradable synthetic materials, (c) can be easily manufactured at large scale, (d) can be sterilized and stored over long periods of time as a lyophilized powder, (e) can be easily portable by the military to be used on-demand, (f) can maintain batch-to-batch consistency in clotting function, and (g) beyond facilitating clotting, can also deliver drugs to help wound healing. To this end we have created such a synthetic platelet technology (SynthoPlateTM, US patent 9107845) using biocompatible and biodegradable lipid-peptide conjugates. In the present proposal, we seek to evaluate the point-of-care applicability of this technology for intravenous hemostatic treatment of combat injury-relevant heavy bleeding, as well as, localized protective/healing treatment of combat-relevant wounds in prolonged field care settings, using pig models. Not only will we evaluate these applications of the SynthoPlateTM technology itself, but we will also study the added benefits of combining this with other clot-stabilizing and infection-preventing drugs.

B. Ultimate Applicability. The primary role of platelets is to help blood clotting. Beyond clotting, platelets also play very important roles in regulating thrombosis, inflammation and immune response in clot retraction and wound healing. Therefore, a synthetic (artificial) mimic of platelet can become a multi-functional technological asset for the military and civilian application in intravenous transfusion medicine to stop heavy bleeding, as well as, a targeted drug delivery platform to treat/regulate thrombotic, inflammatory or immune response.

C. FY16 PFCRA Focus Area Relevance and Impact. Our research is of high relevance and impact to several focus areas in the FY16 PFCRA. In Focus Area 1, the SynthoPlateTM technology can become an alternative or adjunctive to platelet transfusion for prolonged (over 2-4 hours) field care, specifically, pre-hospital hemostasis in austere military settings. By providing platelet-mimicking functions in stoppage of heavy bleeding while avoiding the availability, portability and contamination issues of platelets, the technology can tremendously benefit hemostatic stabilization of wounded soldiers without immediate access to platelet concentrate. The benefit of the technology can further extend into Focus Areas 2 and 3, where it can become an advanced intravenous or locally administered modality for decreasing bleeding-related morbidity and mortality, as well as, a targeted drug delivery platform for resuscitation and wound stabilization for the military, including in TBI.

D. Potential clinical applications, benefits, risks. Potential clinical application and benefit of the SynthoPlateTM technology is its utilization as an artificial platelet substitute in intravenous or local administration for emergency hemostatic management of traumatic bleeding, as well as, prophylactic management of bleeding risks in hem/onc, surgery and platelet dysfunctions. One potential risk may be its off-target effect in the body (e.g. systemic clotting), which will be assessed and mitigated via our proposed studies.

E. Military and Civilian Patient Benefit. If successful, the technology can significantly resolve the challenges faced by the military regarding platelet-transfusion related hemostatic management of traumatic hemorrhage in battlefield conditions, as well as, significantly advance the treatment modalities in combat wound care. These applications can also extend into the civilian population, since many civilian trauma scenarios can happen in remote locations without easy access to platelet transfusion options (e.g. in locations with ill-equipped community hospitals and no trauma centers nearby). The technology can also become applicable to management of traumatic hemorrhage used by emergency responders in pre-hospital settings. Furthermore, the technology can become a transfusable synthetic platelet substitute for prophylactic management of bleeding risks in hem/onc patients (e.g. cancer patients with bone marrow suppression due to chemo/radiotherapy), pre-surgical conditions (e.g. patients to undergo cardiothoracic or other complex surgeries) and platelet dysfunctions (e.g. patients with congenital or drug-related platelet defects or coagulation disorders).

Recipient's Business Official

Authorized Official: Karen Dunn
Title: Assistant Director, Office of Research Administration
Phone: 216-368-4281

Email: kad73@case.edu
DUNS Number: 077758407

Grants Administration Office

Grants Specialist: Robert Doan
Phone: 301-619-2159
Email: robert.t.doan4.civ@mail.mil

Grants Officer's Representative

Congressionally Directed Medical Research Program Office
Phone: 301-619-7071
Email: rene.k.smith.civ@mail.mil

Applicability

These award specific research terms and conditions are applicable to assistance agreement awards (grants and cooperative agreements) issued by the US Army Medical Research Acquisition Activity (USAMRAA) made with institutions of higher education, hospitals, and other non-profit organizations.

Authorities

This new award is a cooperative agreement made under the authority of 10 U.S.C. 2358.

This award is governed by the guidance in 2 Code of Federal Regulations (CFR) part 200, "Uniform Administrative Requirements, Cost Principles, and Audit Requirements for Federal Awards," as modified and supplemented by the Department of Defense's (DoD) interim implementation found at 2 CFR part 1103, "Interim Grants and Cooperative Agreements Implementation of Guidance in 2 CFR part 200" (79 FR 76047, December 19, 2014), all of which are incorporated herein by reference.*

Provisions of Chapter I, Subchapter C of Title 32, CFR, "DoD Grant and Agreement Regulations," parts 26, 28, 34, 37, and 1125 continue to be in effect and are incorporated herein by reference, with applicability as stated in those provisions.

For nonprofit organizations identified in Appendix VIII to 2 CFR part 200, "Nonprofit Organizations Exempted From Subpart E – Cost Principles," and for subawards to commercial organizations, the cost principles in part 31 of Chapter 1 of Title 48, CFR, "Federal Acquisition Regulation" (FAR), and part 231 of Chapter 2 of Title 48, "DoD FAR Supplement," are incorporated herein by reference, with applicability as stated in those provisions.

*Note that OMB amended 2 CFR 200.110(a) on September 10, 2015, to permit recipients to continue to comply with the procurement standards in previously applicable OMB guidance, rather than the procurement standards in 2 CFR 200.317-200.326, through the end of the two recipient fiscal years that begin on or after December 26, 2014. DoD implemented those previous procurement standards in DoDGARs part 32 (32 CFR part 32) for institutions of higher education, hospitals and other nonprofit organizations and in DoDGARs part 33 (32 CFR part 33) for States and local and Indian tribal governments. If you choose to use those previous procurement standards, rather than the standards in PROC Articles I and II of the DoD R&D General T&Cs, you must document that decision in your internal procurement policies.

Copies of the above can be obtained from:

Office of Management and Budget
EOP Publications Office
New Executive Office Building
725 17th Street, NW, Room 2200

Washington, DC 20503
Telephone: (202) 395-7332
Website: http://www.whitehouse.gov/omb/

Terms and Conditions Incorporated by Reference

The following terms and conditions are incorporated herein by reference:

a. Division III - USAMRAA General Research Terms and Conditions with Institutions of Higher Education, Hospitals, and Non-Profit Organizations (effective February 2017), available at http://www.usamraa.army.mil/index.cfm?ID=12&Type=3.

b. The DoD R&D General Terms and Conditions (July 2016), available at http://www.onr.navy.mil/Contracts-Grants/submit-proposal/grants-proposal/grants-terms-conditions.aspx.

These USAMRAA Award Specific Research Terms and Conditions are in addition to the terms and conditions incorporated above.

Order of Precedence

Any inconsistencies in the requirements of this award will be resolved in the following order:

a. Federal statutes
b. Federal regulations
c. 2 CFR part 200 with amendments, as modified and supplemented by DoD's interim implementation found in 2 CFR part 1103
d. Division II - USAMRAA Award Specific Research Terms and Conditions
e. Division III - USAMRAA General Research Terms and Conditions with Institutions of Higher Education, Hospitals, and Non-Profit Organizations (effective February 2017)
f. DoD R&D General Terms and Conditions (July 2016)

Acceptance of Award

You are not required to countersign this award. In case of disagreement with any requirements in this award, contact the USAMRAA Grants Officer in order to resolve the issue(s). Do not assess any costs to the award or accept any payments until the issue(s) is resolved. Note, however, that initiating performance under this award constitutes acceptance of this award, including the terms and conditions.

Catalog of Federal Domestic Assistance Number: 12.420 - Military Medical Research and Development

Statement of Work and Budget

The revised Statement of Work dated 21 September 2021 and the revised budget dated 15 September 2017 for your application submitted in response to the Fiscal Year 2016. DoD Defense Medical Research and Development Program, JPC-6/Combat Casualty Care Research Program Prolonged Field Care Research Award Program Announcement (Funding Opportunity Announcement Number W81XWH-DMRDP-CCCRP-PFCRA which closed 18 August 2016) are incorporated herein by reference.

Recipient's Indirect Cost Rate at the Start of the Performance Period: 60%

Funding Overview

	Federal funds	Cost Sharing	Total amount
Obligated or deobligated this action		N/A	
Cumulative obligations to date, including this and previous actions		N/A	
Planned project costs in the currently approved budget through the end of the period of performance, to include any future incremental funding obligations		N/A	
Total value, which includes any unexercised options for which amounts were established in the award		N/A	

DIVISION II – AWARD SPECIFIC RESEARCH TERMS AND CONDITIONS

TABLE OF CONTENTS

AWARD SPECIFIC TERMS AND CONDITIONS

1. Award Type

This is a cost-type award in support of basic or applied research. Construction activities under this award are not authorized. (Reference Department of the Army Pamphlet 420-11, dated 18 March 2010, for additional information regarding construction activities.)

2. Award Modification

The only method by which the award may be modified is by a formal, written modification signed by the USAMRAA Grants Officer. No other communications, whether oral or in writing, are valid to change the terms and conditions of this award.

3. Maximum Obligation

The maximum obligation of the Federal Government for support of this award will not exceed the award amount specified in the award cover pages, as modified. This award will not be modified to provide additional funds for

such purposes as reimbursement for unrecovered indirect costs resulting from the establishment of final negotiated rates or for increases in salaries, fringe benefits, changes in exchange rates, or other costs. You may rebudget allowable costs in accordance with applicable cost principles and in accordance with the prior approval requirements as stated in this award.

4. Expiration of Funds

(a) Funds obligated on this award are available for use for a limited period based on the fiscal year (FY) of the funds. That time is considered when establishing your period of performance. **This award is funded with FY16 funds which will expire for use on September 30, 2022.** If the final budget period of this award has expiring funds and you do not anticipate expending the total amount by the end of the period of performance, six months before the end of the period of performance contact the Grants Specialist identified in the cover pages of this award**.**

(b) It is extremely important that you monitor the established milestones, timelines, expenditures and invoicing to make sure the project is on schedule and that you voucher promptly. **If this award has funds that will expire on September 30, 2022, submit the final SF270 at least 30 days before September 30, 2022 in order to allow sufficient time to process and pay the voucher.** If you have not submitted a final SF270 and been paid before the expiration date of these funds, any excess funds will be deobligated from the award at that time.

5. Fixed-Amount Awards and Fixed-Amount Subawards

You are not authorized to treat this award or any subawards that you enter into under this award, at any tier, as fixed-amount awards. The inherently unpredictable nature of basic and applied research makes it rarely, if ever, possible to define specific research outcomes in advance, which makes fixed-amount awards inappropriate for research. This is not applicable to procurement contracts entered into under this award for acquisition of supplies, equipment, or general support services you need to carry out the project or program.

6. Prior Approval Requirements

You must request prior approval from the USAMRAA Grants Officer for any of the following program or budget revisions:

a. A change in the scope or objective of the project or program under the award, even if there is no associated budget revision that requires our prior approval.

b. A change in a key person(s) identified in the cover pages of the award.

c. The approved principal investigator's (PI) or project director's disengagement from the project for more than three months, or a 25 percent reduction in his or her time devoted to the project.

d. The inclusion of direct costs that require prior approval in accordance with the applicable cost principles, as identified in FMS Article III of the DoD R&D General Terms and Conditions (July 2016). Note the following requirements and limits:

(1) In accordance with applicable cost principles, you must request prior written approval for the incurrence of special or unusual costs.

(2) The requirement for prior written approval of capital expenditures for equipment that is to be used primarily in carrying out the project or program supported by the award is waived for equipment with a unit cost of $25,000 or less. Capital expenditures for equipment with a unit cost over $25,000 require the USAMRAA Grants Officer's prior approval. **Note that equipment acquired under the award and charged as direct project costs must be necessary for the conduct of the research project supported by the award. You are prohibited from acquiring equipment under the award merely for the purpose of using unobligated balances.**

e. A subaward to another entity under which it will perform a portion of the substantive project or program under the award if it was not included in the approved budget. This does not apply to your contracts for acquisition of supplies, equipment, or general support services you need to carry out the project or program.

f. The transfer (relocation) of the PI and/or research project to another entity.

g. Reimbursing a DoD Military Treatment Facility (MTF) for costs incurred if the MTF is involved in the award. Reimbursing these costs is generally prohibited and only approved under unusual and extraordinary circumstances.

h. Any change in the cost sharing or matching you provide under the award that is included in the approved budget.

i. The need arises for additional Federal funds to complete the project or program.

7. Title to Property

Property acquired in whole or in part with award funds is considered to be excepted property. As such, title is vested to you without further obligation to the Federal Government. Reference PROP Article IV of the DoD R&D General Terms and Conditions (July 2016).

8. Financial Reporting Requirements

a. You must submit Standard Form (SF) 425, "Federal Financial Report," for reporting on this award. Annual and final reports are required.

b. The Federal Financial Reporting period end dates fall on the end of the calendar year for annual reports (12/31), and the end date of the term of award for the final report. Submit annual reports no later than 90 days after the end of the calendar year. Submit final reports no later than 120 days after the end of the period of performance.

c. Submission Instructions:

(1) All SF425 reports must be submitted electronically through the web site https://www.usamraa.army.mil/pages/sf425. The form and instructions can be obtained on this site.

(2) Do not report multiple awards on one report. Each award must be reported separately on its own SF425.

(3) Do not combine multiple SF425s into one submission. Each form must be saved as a separate PDF and submitted individually.

9. Patents and Inventions Reporting Requirements

a. iEdison and annual reporting. You must electronically file Invention Disclosures and Patent Applications using the Interagency Edison (iEdison) system through the National Institutes of Health (https://s-edison.info.nih.gov/iEdison) within the times specified for reporting. In addition, you must report annually any inventions made during the year (within 30 days of the anniversary date of the award) on a DD Form 882, "Report of Inventions and Subcontracts." If there are no inventions during the year, no annual DD Form 882 is required. The DD Form 882 can be accessed at https://www.usamraa.army.mil.

b. Closeout report. A final DD Form 882 is required, whether or not you are reporting an invention. Submit the report within 120 days of end of the period of performance. List all inventions made during the period of performance or state "none," as applicable. The award will not be closed until you have met all reporting requirements.

c. Submit all DD882 reports electronically to usarmy.detrick.medcom-usamraa.mbx.aa4 @mail.mil.

10. Technical Reporting Requirements

The following technical progress reports are required under this award:

Quarterly Technical Reports

a. For each year of the award, the PI must submit Quarterly Technical Progress Reports covering research results (positive and negative data) over a three month period (quarter). A reporting quarter begins with the start date of the award and restarts annually from that date for the entire period of performance. A Quarterly Technical Progress Report for the fourth quarter each year is not required, as the Annual Technical Report must incorporate all four quarters of progress.

b. Quarterly reports are the most immediate and direct contact between the PI and the Grants Officer's Representative (GOR). The reports provide the means for keeping the US Army Medical Research and Materiel Command (USAMRMC) advised of developments and problems as the research effort proceeds. The reports also provide a measure against which funding decisions are made.

c. Prepare all Quarterly reports in accordance with the Quarterly Technical Progress Report format, available at http://www.usamraa.army.mil/index.cfm?ID=12&Type=3. Each item of the report format must be completed.

d. Each report must be submitted electronically, within 15 days after the end of each quarter, to the Grants Specialist and the GOR at the e-mail addresses specified in the front pages of this award. Name your file with your award number, followed by Year X Quarter Y Report (example: W81XWH-17-1-0000 Year 1 Quarter 1 Report.) If you have questions, contact the GOR.

e. Special Reports

Quad Charts: The Quad Chart (available on https://www.usamraa.army.mil) must be updated and submitted as an appendix.

Annual/Final Technical Reporting Requirements

a. Annual Reports

(1) Annual reports are required and must be prepared in accordance with the Research Performance Progress Report (RPPR). The RPPR is the uniform format for reporting performance progress on Federally-funded research projects and research-related activities.

(2) Annual reports must provide a complete summary of the research results (positive or negative) to date in direct alignment to the approved Statement of Work (SOW). The importance of the report to decisions relating to continued support of the research cannot be over-emphasized. An annual report must be submitted within 30 calendar days of the anniversary date of the award for the preceding 12 month period. If the award period of performance is extended by the USAMRAA Grants Officer, then an annual report must still be submitted within 30 days of the anniversary date of the award. A final report that describes the entire research effort is due upon completion of the extended performance date.

b. Final Reports. A final report must also be prepared in accordance with the RPPR and must be submitted within 120 calendar days of the end of the period of performance. The report must summarize the entire research effort, citing data in the annual reports and appended publications.

c. Prepare the annual and final reports in accordance with the RPPR format, available at http://www.usamraa.army.mil/index.cfm?ID=12&Type=3. Although there is no page limitation for the reports, each

report must be of sufficient length to provide a thorough description of the accomplishments with respect to the approved SOW.

d. Reports, in electronic format (PDF or Word file only), must be submitted to https://ers.amedd.army.mil.

Additional information is available on the Researcher Resources website, available at https://mrmc.amedd.army.mil/index.cfm?pageid=researcher_resources.technical_reporting

e. Special Reports

Quad Charts: The Quad Chart (available on https://www.usamraa.army.mil) must be updated and submitted as an appendix.

11. Publication, Acknowledgement, and Public Release

a. Publication. You are encouraged to publish results of the research, unless classified, in appropriate media. Submit one copy of each paper to the GOR simultaneously with its submission for publication. Forward copies of all publications resulting from the research to the USAMRAA Grants Officer or Grants Specialist as they become available, even though publication may in fact occur subsequent to the termination date of the award. (See Section C of the DoD R&D General Terms and Conditions for the charging of publication costs incurred after the period of performance.)

b. Acknowledgment. You agree that in the release of information relating to this award such release will include the statements below, as applicable. "Information" includes, but is not limited to, news releases, articles, manuscripts, brochures, advertisements, still and motion pictures, speeches, trade association meetings, and symposia.

(1) "The U.S. Army Medical Research Acquisition Activity, 820 Chandler Street, Fort Detrick MD 21702-5014 is the awarding and administering acquisition office" and;

(2) "This work was supported by the Office of the Assistant Secretary of Defense for Health Affairs through the Combat Casualty Care Research Program Prolonged Field Care Research Award under Award No. W81XWH-17-2-0064. Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the Department of Defense."

(3) "In conducting research using animals, the investigator(s) adheres to the laws of the United States and regulations of the Department of Agriculture."

(4) "In the conduct of research utilizing recombinant DNA, the investigator adhered to NIH Guidelines for research involving recombinant DNA molecules."

(5) "In the conduct of research involving hazardous organisms or toxins, the investigator adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories."

c. Public release. Prior to release to the public, you must notify the USAMRAA Grants Officer and the GOR of the following: planned news releases, planned publicity, advertising material concerning project work, and planned presentations to scientific meetings. This provision is not intended to restrict dissemination of research information; the purpose is to inform the USAMRMC of planned public release of information on USAMRMC-funded research in order to adequately respond to inquiries and to be alert to the possibility of inadvertent release of information which could be taken out of context.

Failure to include the above statements and adhere to the above regulations, when required, may result in loss of funding and/or termination of this award.

12. Payment Requests

Request for Payments - Fully Funded Award

a. Payments. Payments will be made to you upon receipt of a "grant voucher" (used for both grants and cooperative agreements) submitted through the Wide Area Work Flow (WAWF) e-Business Suite in accordance with the Contract Line Item Number (CLIN) structure set forth in this award.

b. Payment requests can be either advance or reimbursement. Select "advance" or "reimbursement" on the grant voucher in WAWF.

c. In order to conserve administrative resources for both parties, you are encouraged to voucher no more frequently than monthly. **Failure to voucher at least quarterly may raise concerns about research progress and the need for continued funding.**

d. For any advance payment request, you should (1) submit the request approximately 10 days before you anticipate disbursing the requested amount for program purposes, and (2) you must provide an explanation regarding the need for the advance. Include your explanation in the "initiator" block under "comments" or attach an explanation under "attachments." Advance payments must be limited to the minimum amount needed to meet your actual, immediate cash requirements for carrying out the purpose of the approved program or project, including direct program or project costs and a proportionate share of any allowable indirect costs. All advances must be approved by the Grants Officer. Grants Officer approval will be through approval of the grant voucher.

e. All payments will be made by Electronic Funds Transfer (EFT) to your institution's financial account listed in the System for Award Management (SAM) (available at https://www.sam.gov). Failure to update SAM ensuring active account status will result in nonpayment.

f. Failure to submit required Technical Reports or Federal Financial Reports (SF425s) may delay payments or result in nonpayment.

g. If you fail to perform, the grant voucher will be rejected.

h. Interest Bearing Account. You must deposit all advance payments into an interest bearing account unless one of the following applies:

1. You are exempted by applicable Treasury-State agreements in accordance with the Cash Management Improvement Act (31 USC 3335).
2. You receive less than in Federal awards per year.
3. The best reasonably available interest bearing account would not be expected to earn interest in excess of $500 per year on Federal cash balances.
4. The depository would require an average or minimum balance so high that it would not be feasible within the expected Federal and non-Federal cash resources.

i. Interest over the amount of $500 per year must be remitted annually to the U.S. Department of Health and Human Services, Payment Management System, P.O. Box 6021, Rockville, Maryland 20852. A copy of the transmittal letter stating the amount of interest remitted must be sent electronically to usarmy.detrick.medcom-usamraa.mbx.aa4@mail.mil.

13. Electronic Payment Instructions

a. The Wide Area Work Flow (WAWF) e-Business Suite is the required method to electronically process your requests for payments. Once on the WAWF e-Business Suite web site, select the Invoicing, Receipt, Acceptance, and Property Transfer (iRAPT) button to electronically submit "grant vouchers" (used for both grants and cooperative agreements). You must (i) register to use WAWF at https://wawf.eb.mil and (ii) ensure an electronic business point of contact (POC) is designated in the System for Award Management (SAM) site at https://www.sam.gov within ten (10) calendar days prior to requesting a payment for this award.

b. Questions concerning specific payments should be directed to the Defense Finance and Accounting Service (DFAS), Indianapolis, at 1-888-332-7366. **You can also access payment and receipt information using the "myInvoice" button in WAWF at** https://wawf.eb.mil. The award number or grant voucher number will be required to inquire about the status of the payment.

c. The following codes and information are required to initiate the grant voucher and assure successful flow of WAWF documents.

TYPE OF DOCUMENT: **Grant Voucher** ***(Used for both grants and cooperative agreements)***

CAGE CODE: **4B566**

ISSUE BY DODAAC: **W81XWH**

ADMIN BY DODAAC: **W81XWH**

INSPECT BY DODAAC: **W81XWH**

ACCEPT BY DODAAC: **W81XWH**

SHIP TO DODAAC: **W81XWH**

LOCAL PROCESSING OFFICE DODDAC: **Not Applicable**

PAYMENT OFFICE FISCAL STATION CODE**: HQ0490 = DFAS Indianapolis**

EMAIL POINTS OF CONTACT LISTING:
INSPECTOR: **usarmy.detrick.medcom-usamraa.mbx.aa4@mail.mil**
ACCEPTOR**: usarmy.detrick.medcom-usamraa.mbx.aa4@mail.mil**
RECEIVING OFFICE POC: **usarmy.detrick.medcom-usamraa.mbx.aa4@mail.mil**
GRANT ADMINISTRATOR: **Leave Blank**
GRANTS OFFICER: **Leave Blank**
ADDITIONAL CONTACT: **usarmy.detrick.medcom-usamraa.mbx.aa4@mail.mil**

14. Closeout Requirements

a. In order to close this award, you must submit the following documents within 120 calendar days of the end of the period of performance:

(1) Final SF425, "Federal Financial Report." Submit to: https://www.usamraa.army.mil/pages/sf425. Form and instructions are available on the web site.

(2) Final Technical Report. Submit to: https://ers.amedd.army.mil. Forms and instructions are available on the web site.

(3) Final DD Form 882, "Report of Inventions and Subcontracts." Submit to: usarmy.detrick.medcom-usamraa.mbx.aa4@mail.mil. Form is available on web site https://www.usamraa.army.mil).

(4) Property Acquired with Award Funds, if applicable. [Reference PROP Article IV of the DoD R&D General Terms and Conditions (July 2016).]

(a) If title to property (equipment and supplies) is excepted property, there is no further obligation to the Federal Government.

(b) If title to equipment under this award is non-excepted property, you must provide a cumulative listing of nonexpendable personal property acquired with award funds. Submit this on your organization's letterhead. Submit to: usarmy.detrick.medcom-usamraa.mbx.aa4@mail.mil.

(c) If title to supplies under this award is non-excepted property, you must submit a statement that: (i) there is, or is not, a residual inventory of unused supplies exceeding $5,000 in total aggregate value; and (ii) if there is, state whether or not the unused items will be needed on other Federally sponsored projects or programs. Submit this on your organization's letterhead. Submit to usarmy.detrick.medcom-usamraa.mbx.aa4@mail.mil.

b. In the event a final audit has not been performed prior to the closing of this award, the Federal Government retains the right to recover an appropriate amount after fully considering the recommendations on disallowed costs resulting from the final audit.

c. You must promptly refund any unspent balances of funds the DoD Component has advanced or paid that is not authorized to be retained by you. Make check payable to the U.S. Treasury and mail to:

USAMRAA
Attn: MCMR-AAP-C
Federal Award Identification No. W81XWH-17-2-0064
820 Chandler Street
Fort Detrick, Maryland 21702-5014

15. Prohibition of Use of Laboratory Animals

Notwithstanding any other terms and conditions contained in this award or incorporated by reference herein, the recipient is expressly forbidden to use or subcontract for the use of laboratory animals in any manner whatsoever without the express written approval of the USAMRMC, Animal Care and Use Review Office (ACURO). Written authorization to begin research under applicable protocol(s) proposed for this award will be issued in the form of an approval letter from the USAMRMC ACURO to the recipient with a copy to the USAMRAA Grants Officer. Furthermore, modifications to already approved protocols require approval by ACURO prior to implementation. For each fiscal year, the recipient must maintain, and upon request from ACURO, submit animal usage information.

Noncompliance with any of these terms and conditions may result in withholding of funds and/or the termination of the award.

The Animal Care and Use Office requirements can be accessed at https://mrmc.amedd.army.mil/index.cfm?pageid=research_protections.acuro.

16. Prohibition of Use of Human Subjects

Research under this award involving the use of human subjects, to include research involving the secondary use of human biospecimens and/or human data, cannot begin until the USAMRMC's Office of Research Protections (ORP) provides authorization that the research may proceed. The USAMRMC ORP will issue written approval to begin research under separate notification to you. Written approval to proceed from the USAMRMC ORP is also required for any subrecipient that will use funds from this award to conduct research involving human subjects.

The USAMRMC ORP conducts site visits as part of its responsibility for compliance oversight. Accurate and complete study records must be maintained and made available to representatives of the USAMRMC as a part of their responsibility to protect human subjects in research. Research records must be stored in a confidential manner so as to protect the confidentiality of subject information.

The recipient is required to adhere to the following reporting requirements:

Submission of substantive modifications to the protocol, continuing review documentation, and the final report as outlined in the USAMRMC ORP approval memorandum.

Unanticipated problems involving risks to subjects or others, subject deaths related to participation in the research, clinical holds (voluntary or involuntary), and suspension or termination of this research by the IRB, the institution, the Sponsor, or regulatory agencies, must be promptly reported to the USAMRMC ORP.

Change in subject status when a previously enrolled human subject becomes a prisoner must be promptly reported to the USAMRMC ORP HRPO.

The knowledge of any pending compliance inspection/visits by the FDA, ORP, or other government agency concerning this clinical investigation or research, the issuance of Inspection Reports, FDA Form 483, warning letters or actions taken by any Regulatory Agencies, and any instances of serious or continuing noncompliance with regulatory requirements that relate to this clinical investigation or research, must be reported immediately to the USAMRMC ORP.

Non-compliance with these terms and conditions may result in withholding of funds and/or the termination of the award.

DoD requirements for human subjects research, including 32 CFR Part 219, DoD Instruction 3216.02, and USAMRMC ORP Human Research Protection Office submission instructions can be accessed at https://mrmc.amedd.army.mil/index.cfm?pageid=research_protections.hrpo.

17. Prohibition of Use of Human Cadavers

Research, development, testing and evaluation (RDT&E), education or training activities involving human cadaveric specimens under this award shall not begin until approval is granted in accordance with the Army Policy for Use of Human Cadavers for RDT&E, Education, or Training, 20 April 2012 (https://mrmc.amedd.army.mil/index.cfm?pageid=research_protections.overview).

The USAMRMC Office of Research Protections (ORP) is the Action Office (usarmy.detrick.medcom-usamrmc.other.hrpo@mail.mil) for this policy. Approval must be obtained from the USAMRMC ORP. Award recipients must coordinate with the supporting/funding Army organization to ensure that proper approvals are obtained. ORP will issue written approvals to begin under separate notification to the recipient. Written approval to proceed from the USAMRMC ORP is also required for any subrecipient that will use funds from this award to conduct RDT&E, education or training involving human cadaveric specimens.

Recipients must promptly report problems related to the conduct of the activity involving cadavers or the procurement, inventory, use, storage, transfer, transportation, and disposition of cadavers to the USAMRMC ORP.

Recipients must maintain complete records of the activity.

The USAMRMC or designees must be permitted to observe the activity upon request and/or audit activity records to ensure compliance with the approved protocol or applicable regulatory requirements.

Non-compliance with these terms and conditions may result in withholding of funds and/or the termination of the award.

18. Cooperative Agreement: DoD Substantial Involvement

This award is a cooperative agreement due to the anticipated substantial involvement of DoD in performance of the project. In addition to the normal stewardship activities (i.e., site visits, program reviews, performance reporting, and financial reporting activities), it is anticipated that DoD may be substantially involved through collaboration, participation, or intervention in the research to be performed under the award. DoD may become directly involved in performing the research, managing the effort, and/or reviewing and providing approval before work can proceed. The following USAMRMC laboratory(s) or other DoD participant(s) are anticipated to have substantial scientific and/or programmatic involvement: United States Army Institute of Surgical Research (USAISR).

19. Abstracts

An abstract suitable for publication in the proceedings of one DoD meeting may be requested by the GOR during the period of performance of the award. Instructions for the abstract format and submission will be provided prior to the conference.

(End of Summary of Changes)