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TITLE: Multimodal Approach to Improve Functional Recovery Following Acute and Delayed Peripheral Nerve Injury Repair

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14. ABSTRACT

PURPOSE AND SCOPE: This reseach is directed toward the Armed Forces Institute for Regenerative Medicine III, AFIRM III, efforts into addressing Peripheral Nerve Injuries (PNIs). Specifically, this is research to evaluate the feasibility of a very promising polyethylene glycol "PEG-fusion" therapeutic strategy to dramatically improve outcomes to PNIs from single laceration injury or gap (segmental ablations). Ablation PNIs are particularly common in combat. Military or civilian personnel with ablation PNIs have especially poor recoveries due to: (1) immediate loss of axonal integrity so that action potentials are no longer conducted across the lesion sites; (2) immediate loss of sensation and muscle function in affected limb; (3) Wallerian degeneration of distal axons within 1-3 days; (4) slow and poor recovery of sensation and function that occurs only by 1-2 mm/day outgrowths from proximal axons; (5) atrophy of muscles before reinnervation can occur. Ablation PNIs are currently repaired by suturing (neurorrhaphy) of autografts, donor acellular nerve allografts, or synthetic conduits. Viable-cell nerve transplants in a non-protected host immune environment as alternatives are rapidly rejected because of T cell adaptive responses and/or by innate antigen-independent proinflammatory events, even with immune-suppression and major histocompatibility complex (MHC) matching. This research is directed toward: (1) Optimizing PEG Fusion surgical technique translation through understanding the best large animal nerve models (Porcine and Non-human Primate); (2) Increasing the reliability of PEG Fusion process by means of manipulating surgical coaptation and affecting the local immune environment; (3) Evaluating the best combination of techniques and adaptations to inform our partner human clinical trial.

MAJOR FINDINGS/RESULTS:

We have completed re-negotiation (See Appendix 1 for Renegotiation Narrative) among all parties involved in specific aims 1-4. Through our inter-institutional lead scientist hire, Dr Cathy Yang 02/2021 UTA/RESTOR we are choosing to leverage our current success with existing PRORP work and the Bittner lab historical Peg Fusion development and reliability in order to perform the Rodent basic science in autograft best practices and surgeon skill translation. Her role is vital in technical transition of surgical skill in training my surgeon team for maximumly successful Peg Fusion, given that the technique can require skill and understanding beyond that of research personnel; this has been a sticking point and critique of bringing Peg Fusion out of the bench lab and into human operating rooms. **Dr Yang crafted a PEG Fusion course in April of this year capitalizing on common personnel and an IACUC amendment allowing RESTOR personnel to train while PRORP work was being accomplished, we shaved off months of surgical technique establishment.**

Although, we were a little concerned that work could not start on this grant prior to the SOW and Sub Award renegotiation completion. However, through our joint collaboration on a currently executing PRORP FY 18 grant which is a pilot study for our entire PEG Fusion algorithm, we worked out parallel approaches for the rodent to porcine technique translation and satisfied several of our early specific aims for AFIRM III. **Most importantly we were able to establish that the technique was translatable among institutions, allaying an early fear, and thus opening the path to more important science when revised SOW approved**. Through our historical collaboration with Dr Bittner at UT Austin and joint appointment RESTOR/UT Austin Dr Yang, our team completed study of PNA storage in six different solutions. Can store PNAs in Ca-free hypotonic Normosol for at least 4 days at 4^{o}C. We initiated a research agreement with MAJ (Ret) Julia Nuelle who recently left the USAF in an effort to test Normosol (our best nerve storage solution in rats) against a proprietary nerve storage solution for porcine nerves that can be employed at room temperature. Dr's Bittner, Yang, and Nuelle will be examining these porcine nerves harvested through an IACUC for transplant to find the most efficacious storage solution for our AFIRM III proposed autograft in delayed repair. **Thus, in catastrophic combat injury, we have established that nerve can be transported ex-vivo with the patient for autograft use.**

MILITARY BENEFIT/UNIVERSAL SIGNIFICANCE: The PEG-fusion applied research detailed in this proposal would create a reproducible surgical technique directly translatable to humans sustaining PNIs to provide immediate repair of many nerve axons in an injured peripheral nerve. This PEG-fusion technology would prevent a significant amount of prolonged denervation and subsequent severe disability that is typically appreciated immediately after PNIs. Abrogating or preventing the loss of motor control and sensation provides the longest-term benefit to the patient due to the permanent nature of many PNIs. Successful PEG-fusion protocols for humans would significantly change the standard treatment of acute PNI from as far forward into Prolonged Field Care as the Combat Surgical Hospital in Role 3. Because PEG-fusion must occur before Wallerian degeneration becomes irreversible, PNIs would become emergency conditions that require treatment within 1-3 days, as opposed to the weeks or months that are currently recommended. This would represent a paradigm shift in the treatment of acute PNIs.

We have just established as background while SOW renegotiations working through USAMRAA chains that: (1) PEG Fusion is a mentor-able technique where repetition equals greater axonal success; (2) An autograft nerve can be explanted from a patient and stored for transport and later surgery in a safe surgical environment. These are 2 massive steps toward our goal of a nerve reconstruction technique that can be employed forward and change limb salvage.

15. SUBJECT TERMS

Peripheral nerve injury, axotomy, polyethylene glycol fusion, methylene blue, sciatic nerve repair, neurorrhaphy, Wallerian degeneration, allograft transplantation, immunotolerance

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

PEG Fusion (Poly-ethylene glycol fusion) is the process of cellularly fusing nerve after nerve injury. Most of the nerve injuries from combat or major civilian trauma result in large gaps. Nerve usually heals by a process of Wallerian Degeneration by which a nerve axon above the injury recedes back to its cell body and then re-grows to energize the downstream muscle at a slow 1mm/day. As you can imagine, if a nerve has to heal an average 7cm gap it would take 70 days just to span the gap not allowing for the distance the nerve internal fibers, axons, recede back or the length of the gap from the downstream muscle. In many cases the muscle atrophies irreversibly and the function of the muscle group is lost. PEG Fusion obviates this process by allowing immediate nerve transmission keeping the muscle alive while the nerve completes its healing process. The process for bringing this technology to the operating room in the united states and combat support hospitals in the deployed theatre relies on a responsible translation of science. The simplest techniques involve using PEG Fusion in a single cut model as if a patient lacerated a nerve from a sharp object. This must then be translated to nerve gaps where PEG Fusion is used to repair nerves taking advantage of a nerve graft from a "sacrificial" donor of the patient's own nerve. To achieve this translation our group sought to establish that PEG Fusion could be extrapolated from rodents where it was first created by Dr Bittner at UT Austin, by a group of SAMMC surgeons trained in microsurgical nerve repair. They would then explore pig and finally non-human primate to answer questions in neurobiology and technique before attempting this process on human patients. These questions mainly center around using techniques to optimize the number of fibers available for PEG Fusion, help those fibers heal quickly in the body's own response, and remove the surgical expertise required of normal nerve reconstruction to a deployed team who may not repair nerves as part of their daily job.

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

Peripheral nerve injury, axotomy, polyethylene glycol fusion, methylene blue, sciatic nerve repair, neurorrhaphy, Wallerian degeneration, allograft transplantation, immunotolerance

3. ACCOMPLISHMENTS: What were the major goals of the project?

Background: Peripheral nerve injuries (PNI) can be functionally devastating both for civilians and military personnel. For example, an examination of upper extremity combat wounds in Operation Iraqi Freedom and Operation Enduring Freedom revealed that 26% of cases involved a PNI requiring surgery and 84% still experienced residual weakness22-24

The membrane fusogen polyethylene glycol (PEG) has been studied in the restoration of lost behavioral functions when viable host axons at the proximal and distal ends of a cellular nerve graft are fused in a process known as PEG fusion.1,4-11,13,18-21 PEG-fusion has been demonstrated by this consortium's senior partner (George Bittner, UTA) and several collaborators (Bittner, Shores, Weitzel, Alderete, Trevino) to provide dramatic improvement to the speed and quality of recovery from single transection nerve injuries in preclinical animal models. In a small animal model, animals with single cut (SC) nerve transections that were repaired and 5 to 10 mm segmental nerve gaps that were reconstructed with interposition autografts employing PEG fusion exhibit: (1) excellent restoration of sensory and motor functions beginning as early as three days postoperatively; (2) reduction or prevention of Wallerian degeneration20 of axons; (3) reduction or prevention of muscle fiber atrophy or deterioration, and maintenance of NMJ structure and function; and (4) immunoprivileged PEG-fused repair junctions.1,7,10

PEG-fusion of autografts is a new technique that could significantly alleviate many problems produced by segmental nerve loss if it can be successfully translated from a small animal model to clinical practice. This requires further research in both small and large animal models. Our applied research Aims described immediately below are designed to provide data needed for immediate and eventual clinical translation of single transections and ablations repaired by autografts, respectively.

Given the maturity of Dr Bittner's work and the readiness for application towards human clinical use, our research focuses on accomplishing the large task of duplicating Dr Bittner's success in single cut rodent experiments, then transitioning this among 3 institutions, multiple surgeons, and 3 animal models of increasing size and complexity in Peg Fused Autograft Reconstruction (PFAR). Although allograft applications show promise, because we cannot use cellular allograft yet in humans, and allograft use is controversial in contaminated wounds, we are choosing to maximize the translation of autograft because of its lack of alloreactive immune response, greater success than the available "neural tubes" in larger grafts and axons available for fusion.22,27

Optimize Reinnervation: PEG fusion will be studied across a spectrum of animal models and treatment scenarios to improve rapid target reinnervation in PNI. PEG fusion will reestablish continuity of many (but not all axons) and while this is a powerful tool to achieve rapid and clinically significant reinnervation, additional strategies will be explored to enhance the conventional axonal elongation (traditional axonal regeneration) expected to occur in a delayed fashion with PEG fusion.4,8,9 Known axonal growth stimulants (FK506, GNDF, BDNF) will be administered in conjunction with PEG fusion application Rat and Pig.2-4,8,9,24 This will inform the "best case" surgical technique to be used in the non-human primate model and the human clinical trial which is the other funded collaboration for our PEG Fusion team.

SYNERGISTIC VALIDATION OF POLYETHYLENE GLYCOL MEDIATED FUSION (PEG FUSION) AUTOGRAFT RECONSTRUCTION IN LARGE ANIMAL MODEL OF SEGMENTAL NERVE INJURY (SNI)

STATEMENT OF WORK

PROPOSED START DATE – September 15, 2020 | END DATE – September 14, 2024

Site 1	Site 2
San Antonio Military Medical Center	Wake Forest University Health Sciences
PI: Dr. Joseph F Alderete Co-I: Dr. Erik Weitzel	Subaward PI: Vijay Gorantla Subaward Co-I: Fatih Zor

SPECIFIC AIM 1: Rapidly validate the established surgical techniques and reproduce baseline data of PEG-fusion in a rodent model of single cut sciatic nerve injury. Demonstrates inter-institutional transference of PEG fusion treatment strategy.	**Timeline Months**	
Aim 1 will require 120 rats (n=6 for Groups 1, 2 and n=18, for Groups 3-8; 3 time points).	**Site 1**	**Site 2**
Major Task 1: Preparation of studies using PEG-fusion in rat sciatic nerve single cute nerve injury (SCNI) model	1-4	
Subtask 1: Submit documents for IACUC approval for rat and porcine studies	1-2	
Milestone # 1: Obtain IACUC approval for rat, porcine and non-human primate (NHP) studies	3	
Subtask 2: Submit documents for ACURO for rat, porcine and NHP studies	2-3	
Subtask 3: Recruitment/training of personnel; Purchase of reagents/ equipment.	1-6	
Milestone # 2: Obtain ACURO approval for rat and porcine studies	6	
Major Task 2: Assess the effect of the PEG-fusion in rat sciatic nerve SCNI by varying established surgical technique in order to validate a gold standard (best) method	6-18	
Subtask 4: Apply PEG-fusion to the rat sciatic nerve SCNI using standardized epineural repair, with mechanical augmentation fibrin sealant glue (FSG) or microneedle nerve tape (MNT)] for enhancing surgical skills success. 6 groups, total 84 rats. (Table 1, Group 1-6).	6-18	-
Subtask 5: Standard repair + PEG fusion, combined with local augmentation (FK506) in rat sciatic SCNI for improving nerve conductivity and post-procedural function. 2 groups, total 36 rats. (Table 1, group 7-8).	6-18	-
Subtask 6: Perform serial in vivo functional evaluations (catwalk analysis and swim tests) as well as electrophysiologic studies (EMG and nerve conduction) to assess nerve regeneration.	6-18	-
Subtask 7: Perform histopathology, IHC, nerve histomorphometry and genomic evaluations to assess qualitative and quantitative parameters of axonal regeneration	-	6-24

and distal NMJ integrity.		
Milestone # 3: Demonstrate PEG fusion in rat sciatic nerve injury (SCNI) is biocompatible and meet criteria for stability and axonal regenerative capacity	24	
SPECIFIC AIM 2: Apply the above optimized surgical technique and PEG fusion strategy to a rodent nerve transection /autograft repair model for validating the PEG fusion efficiency. Demonstrates translation to PEG fused autograft treatment strategy.	**Timeline Months**	
Aim 2 will require 108 rats (n=18, 3 time points).	**Site 1**	**Site 2**
Major Task 3: Assess the effect of PEG fused autograft in nerve function recovery in a rat nerve gap model with 5mm or 10 mm nerve size gap.	12-24	
Subtask 8: Apply PEG fused autograft in rat SNAI with 5 mm or 10 mm nerve size gap. 4 groups, 18 rats each group. (Table 1, Groups 9-12).	12-24	-
Subtask 9: Apply PEG fused autograft + local neurotherapeutic augmentation (FK506) in rat SNAI with 5mm or 15mm nerve size gap. 6 groups, 18 rats each group. (Table 1, Group 13-14).	12-24	-
Subtask 10: Perform serial in vivo functional evaluations (catwalk analysis and swim tests) as well as electrophysiologic studies (EMG and nerve conduction) to assess nerve regeneration.	12-24	-
Subtask 11: Perform histopathology, IHC, nerve histomorphometry and genomic evaluations to assess qualitative and quantitative parameters of axonal regeneration and distal NMJ integrity.	-	12-24
Milestone # 4: Complete in vivo studies and determine whether PEG fusion of nerve autografts improve functional outcomes in Sciatic Nerve Gap repair model.	24	
SPECIFIC AIM 3: Translate rodent best practice using PEG fusion into viable porcine models of nerve injury with minimal inflammatory cascade and outcomes optimization. Demonstrates translation of PEG fusion strategy to porcine and NHP model.	**Timeline Months**	
Aim 3 will require 36 porcine (n=6, 6 groups) and 10 NHPs (n=2 for control groups and n=3 for experimental groups).	**Site 1**	**Site 2**
Major Task 4: Validate the effect of PEG fusion on functional nerve recovery in porcine forelimb median nerve injury model.	16-36	
Subtask 12: Assess the short-term and long-term effect of PEG fusion in porcine forelimb median nerve single cut injury model. 2 groups, 6 pigs per group. (Table 1, Group 15-16)	16-36	-
Subtask 13: Assess the effect of PEG fused autograft in porcine forelimb median nerve gap injury with 4 cm gap. 2 groups, 6 pigs per group. (Table 1, Group 17-18)	16-36	-
Subtask 14: Assess the effect of PEG fusion combined with local neurotherapeutic augmentation (FK506) in porcine forelimb critical median nerve gap injury (4 cm) model. 2 groups, 6 pigs per group. (Table 1, Group 19-20)	16-36	-
Subtask 16: Perform serial in vivo functional evaluations (Video Gait Analyses) as well as electrophysiologic studies (EMG and nerve conduction) to assess nerve	16-36	-

regeneration.		
Subtask 17: Perform histopathology, IHC, nerve histomorphometry and genomic evaluations to assess qualitative and quantitative parameters of axonal regeneration and distal NMJ integrity.	-	16-40
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Major Task 5: Confirm the effect of PEG fusion on functional nerve recovery in NHP median nerve injury model with long term follow-up.	-	24-44
Subtask 18: Assess the short-term and long-term effect of PEG fusion in NHP forelimb median nerve primary repair model. (Table 1, Group 21 and 22)	-	24-42
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Subtask 21: Perform histopathology, IHC, nerve histomorphometry and genomic evaluations to assess qualitative and quantitative parameters of axonal regeneration and distal NMJ integrity.	-	30-44
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Subtask 22: Data analysis, interpretation of results, comprehensive statistical analysis of study outcomes and final project report for DOD	36-48	
Subtask 19: Final manuscript preparation on entire study.	36-48	
Milestone # 6: Development of a clinically relevant, optimized protocol for PEG-fusion translatable to peripheral nerve injuries following civilian or combat trauma	48	

HJF PI Name:	COL (Dr.) Joseph F Jr Alderete
Collaborator's Name:	**Jaimie Shores MD, The Johns Hopkins University**
Subaward Title:	"Synergistic Validation Of Polyethylene Glycol Mediated Fusion (Peg Fusion) Autograft Reconstruction In Large Animal Model Of Segmental Nerve Injury (Sni)"
Date/Revision #	July 22, 2020; Proposed start date September 2022

Specific Aims 4-5	**Timeline Months: 13-42**	**Site 1 SAMMC** Primary Investigator: Alderete	**Site 2 JHU** Partnering InvestigatorI: Shores
Major Task 1: Execution of Aim 4- Assess the potential short term benefit and improvement in PEG fusion efficacy using mechanical adjuvants for nerve repair in a swine forelimb model.	Begin at month 13 of overall study		
Subtask 1: Finalize surgical and research protocol for swine model related to Aims 4.1-4.3 Participating teams: • Alderete/SAMMC and JHU will share pig protocols to work in parallel and make sure the scope of cross over and independent work is as desired.	13	Dr. Alderete	
Subtask 2: Obtain IACUC and then ACURO approval of animal protocols at JHU	13-15		
Milestone #1: Regulatory approval obtained.	15		
Subtask 3: Execute Aim 4.1-4.3: immediate microsurgical repair of median neurotomy in Yorkshire swine forelimb model: (n=18 animals) 4.1: With and without PEG fusion (n=3+3) 4.2: Utilizing Fibrin Glue Sealant (FGS) on all, with and without PEG fusion (n=3+3) 4.3: Utilizing a novel nerve coaptation device ("Nerve Tape") with and without PEG fusion (n=3+3) • Postoperative observation • Return to OR at 21 days for electrodiagnostic testing, stimulated force generation, and nerve/muscle tissue procurement, terminal exit from study	16-22		

• Histomorphometry and Electron Microscopy: Muscle and nerve biopsy specimens will be evaluated by light, infrared, and electron microscopy for evidence of axonal fusion and persistence, Wallerian Degeneration (WD), myocyte cross sectional mass, motor endplate presence, etc. • Analysis and synthesis of electrodiagnostic, stimulated force, and histomorphologic and electron microscopic results. • Write up and communication of results within consortium.			
Milestone #2: Complete written summary of results and Present at investigators annual meeting and initiate manuscript preparation.	22-24	Dr. Alderete	Dr. Shores
Major Task 2: Execution of Aim 5- Development of a non-human primate model for behavior and functional outcome while evaluating mechanical adjuvants to PEG fusion for improvement in outcome.	Begin at month 25		
Subtask 1: Finalize surgical and behavioral training and assessment research protocol for non-human primate (Rhesus Macaque) model related to Aims 5.1-5.2.	25		Dr. Shores
Subtask 2: Obtain IACUC and then ACURO approvals of protocols at JHU.	25-28		Dr. Shores
Milestone #3: Regulatory approval obtained.	28		Dr. Shores
Subtask 3: Execute Aim 5.1 Removed from Study Plan.	28-37		Dr. Shores
Subtask 4: Execute Aim 5.2 • Train and assess subjects for Kluver pinch board function and volitional grip strength testing. • 5.2 (a) Forearm median neurotomy in randomly chosen (left or right) male Rhesus Macaques with (n=1) and without (n=1) PEG fusion applied. EMG/NCS and stimulated grip strength testing before and after repairs performed. Sham exposure of contralateral arm with testing performed. • 5.2 (b) Forearm median neurotomy in randomly chosen (left or right) male Rhesus Macaques with (n=1) and without (n=1) PEG fusion applied using FGS to mechanically augment the repair of both	29-42		Dr. Shores

subjects. EMG/NCS and stimulated grip strength testing before and after repairs performed. Sham exposure of contralateral arm with testing performed. • 5.2 (c) Forearm median neurotomy in randomly chosen (left or right) male Rhesus Macaques with (n=1) and without (n=1) PEG fusion applied with mechanical augmentation fo the repair site with "Nerve Tape" device. EMG/NCS and stimulated grip strength testing before and after repairs performed. Sham exposure of contralateral arm with testing performed. • 5.2 (d) removed • Histomorphometry and Electron Microscopy: Muscle and nerve biopsy specimens will be evaluated by light, infrared, and electron microscopy for evidence of axonal fusion and persistence, Wallerian Degeneration (WD), myocyte cross sectional mass, motor endplate presence, etc. • Observation of ALL SUBJECTS for 11 months or until plateau of all functional assessments with serial examination every week including behavioral function assessments with modified Kluver pinch board and volitional grip strength testing of bilateral extremities performed, Quarterly MR neurography (MRI) with diffusion tensor imaging (DTI) under anesthesia combined with percutaneous EMG/NCS testing with stimulated grip strength testing of both upper limbs. • At 11 months or the time of functional plateau, all subjects will return to the OR for exposure of the repaired nerve, EMG/NCS testing, stimulated grip strength testing, normal muscle biopsy, median nerve innervated muscle biopsy, nerve biopsy and repair. • Observation for up to 1 month for return of function and socialization skills that would enable discharge into "retirement colony" or return to research colony at large. • Histomorphometry and Electron Microscopy:			

Muscle and nerve biopsy specimens will be evaluated by light, infrared, and electron microscopy for evidence of axonal fusion and persistence, Wallerian Degeneration (WD), myocyte cross sectional mass, motor endplate presence, etc. • Analysis and synthesis of electrodiagnostic, stimulated force, and histomorphologic and electron microscopic results. • Write up and communication of results within consortium.			
Milestone #3: Completion of primate work and written summary of study results for presentation at investigators and initiate manuscript preparation.	42	Dr. Alderete	Dr. Shores

Statement of Work [SOW] *Sub to Dr Margaux Salas*

HJF PI Name:	COL (Dr.) Joseph F Jr Alderete
Collaborator's Name:	Margaux Salas, PhD; METIS
Subaward Title:	"Synergistic Validation Of Polyethylene Glycol Mediated Fusion (Peg Fusion) Autograft Reconstruction In Large Animal Model Of Segmental Nerve Injury (Sni)"
Date/Revision #	July 22, 2020

INTRODUCTION/BACKGROUND:

COL (Dr.) Joseph Alderete is located at The Brooke Army Medical Center, Center for Interpid and conducts research in the field of Orthopaedic Oncology, Trauma, and Adult Reconstruction. He will be performing studies for the Proposal Number DM190618P3 to specifically study the project entitled, "Multimodal Approach to Improve Functional Recovery Following Acute and Delayed Peripheral Nerve Injury Repair". COL (Dr.) Alderete will utilize the technical expertise of Margaux Salas, PhD from the Metis Foundation to meet the objectives identified in the above mentioned study. Specifically, Dr. Salas will perform the serial in vivo functional evaluations (catwalk analysis and swim tests) as well as electrophysiologic studies (EMG and nerve conduction) to assess nerve regeneration.

Dr. Salas will train, supervise and complete serial *in vivo* functional evaluations (catwalk analysis and swim tests) as well as electrophysiologic studies (EMG and nerve conduction) to assess nerve regeneration. Dr. Salas will supervise and oversee all animal work accomplished within the RESTOR laboratory and will also contribute to all regulatory approvals and quarterly reports. Dr. Salas will analyze and assess all animal outcomes quarterly to assure best practices and quality control of data acquired (including histopathology, IHC, nerve histomorphometry and genomic evaluations).

Specific Aim 1(specified in proposal)	**Timeline**	**Site 1**
Major Task 2	Months	
Subtask 6: Perform serial in vivo functional evaluations (catwalk analysis and swim tests) as well as electrophysiologic studies (EMG and nerve conduction) to assess nerve regeneration.	6-18	Dr. Salas
Specific Aim 2		
Major Task 3		
Subtask 10: Perform serial in vivo functional evaluations (catwalk analysis and swim tests) as well as electrophysiologic studies (EMG and nerve conduction) to assess nerve regeneration.	12-24	Dr. Salas

What was accomplished under these goals?

Specific Aim 1:

We have completed re-negotiation (See Appendix 1 for Renegotiation Narrative) among all parties involved in specific aim 1. We are choosing to leverage our current success with existing PRORP work and the Bittner lab historical Peg Fusion development and reliability in order to perform the Rodent basic science in autograft best practices and surgeon skill translation. We have hired Dr Cathy Yang PhD as Peg Fusion scientific advisor who is the lab manager and neuroscientist for Dr Bittner at UT Austin. Her role is vital in technical transition of surgical skill in training my surgeon team for maximumly successful Peg Fusion, given that the technique can require skill and understanding beyond that of research personnel; this has been a sticking point and critique of bringing Peg Fusion out of the bench lab and into human operating rooms. Dr Yang crafted a PEG Fusion course in April of this year capitalizing on common personnel and an IACUC amendment allowing RESTOR personnel to train while PRORP work was being accomplished, we shaved off months of surgical technique establishment.

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Through our historical collaboration with Dr Bittner at UT Austin and joint appointment RESTOR/UT Austin Dr Yang, our team completed study of PNA storage in six different solutions. Can store PNAs in Ca-free hypotonic Normosol for at least 4 days at 4^{o}C. We initiated a research agreement with MAJ (Ret) Julia Nuelle who recently left the USAF in an effort to test Normosol (our best nerve storage solution in rats) against a proprietary nerve storage solution for porcine nerves that can be employed at room temperature. Dr's Bittner, Yang, and Nuelle will be examining these porcine nerves harvested through an IACUC for transplant to find the most efficacious storage solution for our AFIRM III proposed autograft in delayed repair. **Thus, in catastrophic combat injury, we have established that nerve can be transported ex-vivo with the patient for autograft use.** We continue to expound upon our experience with previous PRORP grant that serves as a pilot study for AFIRM III. Specifically, we have engineered 3 papers on the porcine model for Peg Fusion Autograft and Allograft as well as continued to push collaborative efforts with the Bittner UT Austin Lab looking at the immunogenicity of Peg Fusion because it causes such a robust inflammatory response by itself.

Specific Aim 2:

We elucidated the most reproduceable and fastest surgical approach to the porcine forelimb for single cut and segmental gap reconstruction which became the source of multiple revisions to our AFIRM III IACUC protocol for animal safety and utility. Finally, we were able to serendipitously determine that ulnar nerve cable graft to median nerve would facilitate a "gold standard" autograft for use in AFIRM III autograft studies because the mixed motor and sensory nerve graft would have the highest potential behavioral response after reconstruction. Unfortunately, in an effort to continue our good relationship with our porcine veterinary team at 59th Medical Wing, each time we discovered a major surgical technique jump we were forced to engineer another IACUC amendment for AFIRM III experimnets.

Dr, Alderete was accepted as a Co-Editor of an issue of Frontiers in Cellular Neuroscience dedicated to topic of **Restoring Function After Traumatic Peripheral Nerve Injury**With Drs. Bittner and Shores in various combinations, we submitted a DOD PRORP grant, a DOD multi institutional RTRP grant and an NIH multi-institutional R-01 grant to use the data obtained on this PRORP and transition toward clinical trials.

Study of the effects of methylene Blue (MB) on PEG-fusion repair of single transections (Specific Aim 2):

Ghergherehchi CL, Shores JT, Alderete J, Weitzel EK, Bittner GD. 2021. Methylene blue enhances PEG-fusion repair of completely severed rat sciatic nerves. Neural Regeneration Research. doi.org/10.1186/s12974-020-01953-8.

Review articles on the immunotolerance of Peg Fusion (*Will go a tremendously long way in understanding human autograft and allograft segmental nerve reconstruction in Peg Fusion*)

Tyler A. Smith, Cameron L. Ghergherehchi, Kelly C.S. Roballo, Jared A. Bushman, **Erik K. Weitzel, Jaimie T. Shores, Joseph A Alderete,** Michelle Mikesh, Haley O. Tucker, **George D. Bittner. 2021**. Polyethylene glycol treatment of peripheral nerve allografts without axonal fusion diminishes T cell infiltration and MHC expression, but does not prevent Wallerian degeneration-associated cellular responses J. Neuroinflammation. September 2021 submission.

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

If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

We have 3 additional Manuscripts in preparation for the early Porcine Model work of this endeavor:

1) Rask, D. Yang, C. Bernal, A. Cox, J. Salas, M. Shores, J. Bittner, G. Alderete, J. "Injured Nerve Preparation in Neurrhapy; examination of axon damage in 11, 10, and 15 blade sharp transection before repair." Manuscript in progress.
2) Rask, D. Yang, C. Bernal, A. Cox, J. Salas, M. Shores, J. Bittner, G. Alderete, J. "A Porcine Forelimb Model is the Optimal Peripheral Nerve Trainer: Anatomy, Recovery, and Clinical Translation". Manuscript in progress.
3) Rask, D. Yang, C. Bernal, A. Cox, J. Salas, M. Shores, J. Bittner, G. Alderete, J. "Median and Ulnar Nerve Ultrastructure in a Porcine Model of Peripheral Nerve Repair. Manuscript in progress.

Finally, we have submitted an abstract for Extremity War Injury Symposium 2021: Rask, D. Yang, C. Bernal, A. Cox, J. Salas, M. Shores, J. Bittner, G. Alderete, J. Peripheral Nerve Injury: PEG Fusion and Closer to Skywalker. Submission in review. Washington DC 2021.

Casey Sabag MD, part of our hand surgery team at BAMC, while be using Magnetic Resonance Neurography in PEG Fusion Neurrhaphy as her thesis for SCION Clinical Research Fellowship out of the grant work.

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

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

We have disseminated early experience with the exploration of knowledge gaps by incorporating PEG Fusion discussion into the Advanced Microsurgery Course while we are working out the best methods to teach the technique with Dr Yang at UT Austin. This course is a cognitive and skills development for military and civilian microsurgeons in conjunction with UT San Antonio. Once we are into the neuromodulation and technique coaptation described into our SOW we will start teaching PEG Fusion technique for later use in the clinical trial with Dr Shores.

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "Nothing to Report."

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

I hope to find in our next reporting period that we have received approval for SOW amendment and renegotiation and can proceed with rodent experiments at UT Austin. Once this is underway we can begin to parallel fight out the best practices in immunomodulation (FK506, methylprednisolone) and technique adaptation (fibrin sealant glue, microhook nerve tape, etc.) and translate back and forth from rodent to porcine in a now well established work flow between UT Austin and RESTOR. This year I hope to understand contributions of epineurium and cable grafting to number of axons available for PEG Fusion as we seek to optimize results before non-human primate work.

4. **IMPACT:** D*escribe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

Due to advances in body armor and battlefield medicine, wounded warriors are surviving more injuries and living with the sequelae of major extremity trauma, including peripheral nerve injuries (PNIs). Major PNI is a continued source of permanent disability. This permanent disability is documented in two recent military medicine studies. In one report, 32 patients with open tibial fractures had 43 PNI's[1]. In a second report, 189 soldiers evacuated for upper limb injuries and 70 had PNIs that were the main cause of their disability[2].
The losses due to PNI can be staggering: individual disability and inability to return to work rates are high even after repair/reconstruction. In civilian populations, a combined PNI in the upper extremity results in return to work of <25% at any time point. From the standpoint of military medicine's obligation to our wounded and injured soldiers and sailors, and the ability to maintain military readiness, PNI takes a heavy toll.

While many strategies are currently being researched to accelerate and/or enhance nerve regeneration after PNI, PEG-fusion, especially of allografts, is the only feasible strategy of which we are aware of that might eliminate the need for nerve regeneration by slow axonal regrowth that often takes many months, if ever, to reach target tissues that may have atrophied. That is, successful PEG-fusion repair of some nerve axons in a peripheral nerve prevents their Wallerian degeneration, provides immediate re-innervation, and rather rapid (weeks) sensory and motor recovery of function. Thus, PEG-fusion of allografts presents an opportunity to prevent one of the largest forms of disability (and inability to return to duty/employment) that occur in major extremity trauma.

To enable translation of this technology to human use, we have chosen 2 animal models. The traditional rat model, which most peripheral nerve research has been established in, and within which we have significant PEG-fusion research experience already, will be used for the preliminary phase of the study to help establish timelines for translation to our larger porcine model. We have significant experience using swine for animal models in other areas of research within our respective clinical groups as it is the large animal model of choice for research in vascularized composite allotransplantation and is frequently used for the study of shock/trauma in military medical training and research. This is due to their larger size, immune system and physiological similarities to humans, and the conserved anatomy that is directly applicable to humans. Another reason that the porcine model is ideal for this study is the larger caliber of forelimb nerves (3-5 mm diameter). As swine anatomy is very similar to humans, their nerve size is consistent with humans compared to smaller animals (0.5 mm in rats versus 3-5 mm in the swine forelimb) which will allow for greater translation at the conclusion of this study.

Immediate impact:
The PEG-fusion applied research detailed in this proposal would create a reproducible surgical technique directly translatable to humans sustaining PNIs to provide immediate repair of many nerve axons in an injured peripheral nerve. This PEG-fusion technology would prevent a significant amount of prolonged denervation and subsequent severe disability that is typically appreciated immediately after PNIs.

Long-term impact:
Two categories of long-term impact require discussion. The first is the individual long-term impact to the wounded warrior. Prevention of Wallerian degeneration, which is programmed to occur in injured nerves, leads to loss of motor control and muscle atrophy that are difficult, if not impossible, to reverse depending on the injury. Sensory loss is also disabling as a person is unable to protect the insensate portion of their body that is further prone to injury and less useful due to lack of sensory feedback. Abrogating or preventing the loss of motor control and sensation provides the longest-term benefit to the patient due to the permanent nature of many PNIs.

The second category of long-term impact is on health care systems and delivery. Successful PEG-fusion protocols for humans would completely change how acute PNIs are treated. Because PEG-fusion must occur before Wallerian degeneration becomes irreversible, PNIs would become emergency conditions that require treatment within 1-3 days, as opposed to the weeks or months that are currently recommended. This would represent a paradigm shift in the treatment of fresh, acute PNIs.

The effect upon Prolonged Field Care (PFC) would also be two-fold: Preparation of a PNI to try and potentially prolong its ability to undergo PEG-fusion, and evacuation in a timely manner to a surgical site capable of performing PEG-fusion within an acceptable period of time would need to occur. Both of these changes to PFC would optimize the ability for patients to undergo

What was the impact on other disciplines?

Healthcare needs of the Military

As previously stated, PNIs are common in extremity traumas associated with combat casualties. In addition, PNIs are frequent in civilian populations with more common mechanisms of injury such as motor vehicle accidents, sports injuries, accidental traumas, etc., ---- all of which ALSO affect active duty and reserve military personnel. In addition to the military studies already discussed, the U.S Health insurance group estimated that an annual incidence of 67,800 major PNIs in the U.S.[4] A commercial market report by Brattain[5] estimates a substantially larger number of annual PNIs (450,000 – 660,000). These PNIs create a large disability cost in both civilians and in current and potential military personnel from combat and non-combat related injuries. This study addresses acute simple nerve injuries (single cut) as well as larger segmental "ablation" injuries (allograft). The strategy for treating ablation injuries in this study is different from the standard use of "autograft", which requires the use of a nerve from a less critical part of the body to reconstruct a nerve gap in a more critical part of the body. A technique that creates an additional donor morbidity with the hopes of regaining a portion of the original critical limbs function. Instead, this study focuses upon reconstruction of segmental nerve injuries using "allograft" nerve, or nerve taken from a separate donor. The reasons this study is focusing on allograft instead of autograft is that the most severely injured of our wounded warriors typically have multi-extremity debilitating trauma. This makes obtaining autograft to reconstruct their injuries unrealistic. Not only may they not have intact donor sites to take nerve from, but the added amount of time required to obtain donor nerve with additional incisions and morbidity on potentially already compromised limbs, or even take further sensation away from their only remaining intact limbs, are all current suboptimal options. This study looks to try and overcome the challenge of inadequate "replacement" nerve by attempting to utilize nerve allografts so that no increased iatrogenic morbidity occurs to the already injured soldier. The PEG-fusion allograft is a technique that will, in addition, provide a treatment that alleviates limitations on what can be reasonably reconstructed based on available donor sites. While the need for viable allograft with living cells creates a second challenge in the treatment of these patients, it is not a challenge that cannot be overcome. This study also evaluates strategies to prolong the amount of time that a nerve injury can wait before repair/reconstruction as well as the amount of time a nerve allograft may be kept in preparation for use in the reconstruction of these devastating injuries.

1) Beltran MJ, Burns TC, Eckel TT, Potter BK, Wenke JC, et al. (2012) Fate of combat nerve injury. J Orthop Trauma 26: e198-203.
2) Rivera JC, Glebus GP, Cho MS (2014) Disability following combat-sustained nerve injury of the upper limb. Bone Joint J 96-B: 254-258.
3) Bruyns CN, Jaquet JB, Schreuders TA, Kalmijn S, Kuypers PD, et al. (2003) Predictors for return to work in patients with median and ulnar nerve injuries. J Hand Surg Am 28: 28-34.
4) Taylor CA, Braza D, Rice JB, Dillingham T (2008) The incidence of peripheral nerve injury in extremity trauma. Am J Phys Med Rehabil 87: 381-385.
Brattain, K. Analysis of the peripheral nerve repair market in the United States. Magellan Market Report. Magellan Medical Technology Consultants, Inc.

What was the impact on technology transfer?

PEG-fusion has been demonstrated to provide dramatic improvement to the speed and quality of recovery from single transection nerve injuries in preclinical animal models. Comparable results have also been produced in pilot human clinical cases. Preliminary results for the use of PEG-fusion for allo- and autograft repair of segmental ablation PNIs indicate similarly dramatic outcomes. The potential for PEG-fusion is high in acute injury nerve repairs and several other surgical procedures involving peripheral nerve reconnections and reconstructions.

Neuraptive Therapeutics, Inc., has licensed the PEG-fusion technology from UTA and incorporated it into a commercially viable surgical product called AxoFuse. The work proposed herein will support advancement of PEG-fusion for graft repairs into clinical studies and ultimately FDA approval and commercial use of AxoFuse. The Company has raised over $14M to conduct clinical studies of AxoFuse for acute nerve injury repairs and advance this promising technology towards clinical use. Neuraptive intends to bring AxoFuse to market for use in a variety of clinical applications including those resulting from the studies outlined in this grant proposal.

Beyond the work described herein, additional preclinical and clinical efforts are underway to establish the utility for AxoFuse in live nerve coaptation for high-value surgical applications including targeted muscle reinnervation, autologous tissue transfers (e.g. breast reconstruction using tissue flaps), facial reanimation, limb replantation, and ultimately limb and face transplantation.

AxoFuse has been presented to the FDA's Division of Neurological Products (DNP) within the Center for Drug Evaluation and Research (CDER). FDA has agreed with the product composition and design as well as the appropriateness of AxoFuse for clinical studies in acute nerve injury repairs. AxoFuse is being manufactured in an FDA-compliant facility and was presented for formal approval to conduct clinical studies under an FDA-approved, company-sponsored Investigational New Drug (IND) in 2020. This approval has recently been granted. Furthermore, in collaboration with UUTA, JHU and RESTOR/Metis have recently been awarded an AFIRM grant for about $6M to conduct additional translational studies on swine, monkeys, and a clinical trial.

Nerve repair surgeons have been particularly receptive to AxoFuse as there have been no major advances in nerve repair since the advent of neurorrhaphy in World War II. In addition, as the compounds in the AxoFuse solutions are all USP-grade and have been in widespread human use for decades, there is a very low safety risk as recognized by both surgeons and FDA. The drug delivery device in the AxoFuse kit is made of medical-grade silicone, is not implanted, and presents no risk to patients. Clinical development of AxoFuse will follow FDA guidance. The first trial of AxoFuse will be for acute repair of single cut injuries and should commence in 2022.

What was the impact on society beyond science and technology?

Successful PEG-fusion protocols for humans would completely change how acute PNIs are treated. Because PEG-fusion must occur before Wallerian degeneration becomes irreversible, PNIs would become emergency conditions that require treatment within 1-3 days, as opposed to the weeks or months that are currently recommended. This would represent a paradigm shift in the treatment of fresh, acute PNIs.

5. CHANGES/PROBLEMS: *The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:*

Please see appendices for AFIRM III award renegotiation documents. I anticipate this will only set us behind by 3 months when given the green light as we are poised to execute multiple arms and courses of action in parallel to satisfy work requirements.

I retire from active service in April 2023; however, am making arrangements with USAISR to maintain FTE to complete this study in 2024 and the AFIRM III human clinical trial. My deputy, MAJ Casey Sabbag will execute the DOD chair for this lab upon my retirement as I stay on as science chair.

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

Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

Our renegotiation phase after sub-awards had been decided was a significant setback. With my deployment to Iraq in 2020 our final USAMRAA deliberations took place with me 8 hours away in a combat theatre. This forced us to accept some SOW activity designed to save money without trully considering that one of our most important partners in this translation, Dr George Bittner at UT Austin, who had been unfunded his portion of original AFIRM submission on allograft, was left out of the knowledge translation and almost killed our ladder of evidence. This was the source for our SOW re-negotiation submitted before you now.

Changes that had a significant impact on expenditures

Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

Originally, I did not understand the MIPR and actual budget determinations for early in the grant commencement which definitely affected how quickly I could react to USAMRAA budget acceptance and negotiation calls. Luckily our science officer and grant managers were finally able to explain what has become a huge education in programmatic research

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

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

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Significant changes in use or care of human subjects

No changes.

Significant changes in use or care of vertebrate animals

No changes.

Significant changes in use of biohazards and/or select agents

No changes

6. PRODUCTS: *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*

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

Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Study of the effects of methylene Blue (MB) on PEG-fusion repair of single transections (Specific Aim 2):

Ghergherehchi CL, Shores JT, Alderete J, Weitzel EK, Bittner GD. 2021. Methylene blue enhances PEG-fusion repair of completely severed rat sciatic nerves. Neural Regeneration Research. doi.org/10.1186/s12974-020-01953-8.

Review articles on the immunotolerance of Peg Fusion (*Will go a tremendously long way in understanding human autograft and allograft segmental nerve reconstruction in Peg Fusion*)

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Dr, Alderete was accepted as a Co-Editor of an issue of Frontiers in Cellular Neuroscience dedicated to topic of **Restoring Function After Traumatic Peripheral Nerve Injury**

Other publications, conference papers and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

Finally, we have submitted an abstract for Extremity War Injury Symposium 2021: Rask, D. Yang, C. Bernal, A. Cox, J. Salas, M. Shores, J. Bittner, G. Alderete, J. Peripheral Nerve Injury: PEG Fusion and Closer to Skywalker. Submission in review. Washington DC 2021.

Casey Sabag MD, part of our hand surgery team at BAMC, while be using Magnetic Resonance Neurography in PEG Fusion Neurorrhaphy as her thesis for SCION Clinical Research Fellowship out of the AFIRM III grant work.

- **Website(s) or other Internet site(s)**
List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

NA

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

4) Rask, D. Yang, C. Bernal, A. Cox, J. Salas, M. Shores, J. Bittner, G. Alderete, J. "A Porcine Forelimb Model is the Optimal Peripheral Nerve Trainer: Anatomy, Recovery, and Clinical Translation". Manuscript in progress.

5) Rask, D. Yang, C. Bernal, A. Cox, J. Salas, M. Shores, J. Bittner, G. Alderete, J. "Median and Ulnar Nerve Ultrastructure in a Porcine Model of Peripheral Nerve Repair. Manuscript in progress.

- **Inventions, patent applications, and/or licenses**
Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

NA

- **Other Products**

With Drs. Bittner and Shores in various combinations, we submitted a DOD PRORP grant, a DOD multi institutional RTRP grant and an NIH multi-institutional R-01 grant to use the data obtained on this PRORP and transition toward clinical trials.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

Name: George Bittner, PhD

Project Role: PI, UTA

Nearest person month worked: 0.6

Contribution to Project: Initiating PI, coordinating experiments, evaluating production.

Name: Jaimie Shores MD

Project Role: Co-PI, JHU

Nearest person month worked: 0.6

Contribution to Project: Co-PI, coordinating experiments, evaluating production

Name: Col Erik Weitzel MD

Project Role: Co-PI, RESTOR

Nearest person month worked: 0.6

Contribution to Project: Co-PI, coordinating experiments, evaluating production

Name: COL Joseph Alderete MD

Project Role: Co-PI RESTOR/FUSE

Nearest person month worked: 2.5

Contribution to Project: Initiating PI, coordinating experiments, evaluating production

Name: Alejandro Bernal

Project Role: Fellow, RESTOR

Nearest person month worked: 0.6

Contribution to Project: Coordinating experiments, performing experiments, obtaining samples, data collection

Name: Cameron Ghergherehchi, PhD

Project Role: PhD Research Scientist

Nearest person month worked: 0.5

Contribution to Project: Coordinating experiments, performing experiments, obtaining samples, data collection, rat microsurgery

Name: Jennifer Cox

Project Role: Lab Manager

Nearest person month worked: 0.6

Contribution to Project: Study documentation, Data collection, ordering supplies and evaluation of experimental costs.

Name: Cathy Yang, MD,PhD

Project Role: Lab Manager, Research Scientist UTA

Nearest person month worked: 0.8

Contribution to Project: Study documentation, Data collection, ordering supplies, co-ordinating undergraduate animal testing, rat microsurgery

Name: Paul Oliphint, BS

Project Role: Lab Manager

Nearest person month worked: 3.0

Contribution to Project: Study documentation, Data collection, ordering supplies, co-ordinating undergraduate animal testing, performing confocal, fluorescent, TEM studies and analyses

Name: Tyler Smith, PhD

UTA Project Role: PhD, Research Scientist

Nearest person month worked: 2.0

Contribution to Project: Coordinating experiments, performing IHC experiments, obtaining samples, data collection

Name: MAJ Julia Nuelle MD

Project Role: RESTOR Co-I

Nearest person month worked: 0.6

Contribution to Project: Alternate RESTOR PI, coordinating experiments, evaluating production.

Name: Sruja Arya, Mario Carrera, Ted Zhao. Sruja Arya, Rhea Sachdeva,

Project Role: Undergraduate Research Assistants (URAs), UTA

Nearest person month worked. 2.0

Contribution to Project: Animal behavioral testing (all), Carrera, Arya: learning rat microsurgery

Name: MAJ Casey Sabbag MD

Project Role: RESTOR Co-I

Nearest person month worked: 0.6

Contribution to Project: Alternate RESTOR PI, coordinating experiments, evaluating production.

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

Executive officer for RESTOR/FUSE limb Salvage lab MAJ Julia Nuelle MD retired from USAF and took a staff surgeon position with Mizzou. She will serve RESTOR/FUSE on a continued basis as ORISE Clinician Scientist knowledge translator.

What other organizations were involved as partners?

- *Other*.

Organization Name: Johns Hopkins University

Location of Organization: Baltimore, Maryland

Partner's Contribution to the Project: (identify one or more, e.g. 1) financial support; 2) in-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff); 3) facilities (e.g., project staff use the partner's facilities for project activities); 4) collaboration (e.g., partner's staff work with project staff on the project); 5) personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); etc. Assisted with Specific Aim 2, **Translate results and lessons learned in rodent model of single cut and ablation type PNI with respect to timing, allograft storage, and inflammatory modification to a large animal model**

Organization Name: 59th Medical Wing Clinical Research Division: RESTOR/FUSE Clinical Lab

Location of Organization: JBSA Lackland Airforce Base

Partner's Contribution to the Project: Assisted with Specific Aim 2, **Translate results and lessons learned in rodent model of single cut and ablation type PNI with respect to timing, nerve storage, and inflammatory modification to a large animal model**

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: *For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to* ***https://ebrap.org/eBRAP/public/index.htm*** *for each unique award.*

N/A

QUAD CHARTS: *If applicable, the Quad Chart (available on https://www.usamraa.army.mil/Pages/Resources.aspx) should be updated and submitted with attachments.*

Please see attached.

9. APPENDICES: *Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.*

Appendix 1: AFIRM III Renegotiation Documents

1A: Programmatic Justification
1B: MIPR Budget
1C: WFIRM CRO
1D: UTA Budget
1E: JHU Budget

Appendix 2: Longer PNA Review

Appendix 3: Methylene Blue paper