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

PRINCIPAL INVESTIGATOR: COL Joseph F. Alderete, MD, FAOA

CONTRACTING ORGANIZATION: The Henry M. Jackson Foundation for the Advancement of Military Medicine Inc. Bethesda, MD

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COL Joseph F. Alderete, MD, FAOA

E-Mail: joseph.f.alderete.mil@mail.mil

5d. PROJECT NUMBER

5e. TASK NUMBER

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

Brooke Army Medical Center
3551 Roger Brooke Dr.
Fort Sam Houston, TX 78234

HENRY M. JACKSON FOUNDATION
FOR THE ADVA
H J F
6720-A ROCKLEDGE DR STE 100
BETHESDA MD 20817-1891

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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 2 for Renegotiation Narrative) among all parties involved in specific aims 1-4. New SOW approved March 2022. 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 (See Appendix 1) 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. IACUC and ACURO for UTA complete. Bench experiments for Tisseel and FK506 starting with Baxter and Astellas Pharma guidance at 5mg/ml FK506 of variable concentration and clot architecture/porosity (See Appendix 3) Dr Yang developed EMG/NCS PEG Fusion validation teach and ttechnical 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 2022 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 was 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.**

Of immense validation, at American Society of the Hand '22 there were several papers that demonstrated failure of PEG Fusion in demonstrating nerve repair improvement, These studies did not train to standard as we have undertaken and did not electrodiagnostically verify on table fusion as Dr Yang has taught us. Therefore, our training algorithm and accuracy verification will prove invaluable to the science.

As of OCT 2023 IACUC and ACURO for UT Austin complete. IACUC for the Porcine Model through RESTORE/59th UT Austin complete and IACUC pending. NHP IACUC for JHU pending.

SOW Specifics: Aim 1: Obtain multimodal PEG- fusion baseline and Environmental Augmentation data on Sprague Dawley rat sciatic, single cut, nerve model systems for behavioral recovery (SFI, von Frey tests), axonal/NMJ/muscle morphology and function (CAPs, CMAPs).

Aim 1A: Train four surgeons from DOD RESTOR San Antonio to PEG-fuse singly cut rat sciatic nerves assayed by weekly SFI behavioral tests for 6 weeks. 6 rats/surgeon. 24 Sprague Dawley (SD) or Lewis chronic rats. Two surgeons from DOD RESTOR have been trained in UT Austin and two additional surgeons also trained on April 28th 2023; 100%. **Aim 1B:** Baseline Data . PEG-fuse *singly cut* Sprague Dawley or Lewis rat sciatic nerves enhanced by FK506 application assayed by behavioral recovery (SFI, von Frey tests), axonal/NMJ/muscle morphology and/or function (CAPs, CMAPs). Compare to PEG-fused and NC historical data. 15 Sprague Dawley (SD) or Lewis chronic rats. 1: Obtain von Frey test Baseline data (see attached) papers : 80%. 2: Established the tisseel application procedure with the help of the Baxter staff

3: Operated 4 SD rats with only two sutures and tisseel applications. A: Only one of the 4 operated animals recovered with an SFI score better than -69.

B: It appears that the repairs with tisseel had minimal inflammation (or neuroma) without axon sprouting outside of epineural sheaths. For the future surgeries for this Aim, we are planning to perform the traditional neurorrhaphy: at least 4 sutures without tisseel application. C: Only one of 4 animals (25%) had very minor self-mutilation: our historical data in the lab was greater than 50%, indicating that tisseel might have helped prevent from self-mutilation although the number of animals is relatively small to make a concrete conclusion

Aim 2: Baseline data and Environmental Augmentation data. Obtain multimodal PEG- fusion baseline data on Lewis rat sciatic isograft, nerve model systems for behavioral recovery (SFI, von Frey tests), axonal/NMJ/muscle morphology and/or function (CAPs, CMAPs). **Aim 2A.** Baseline Data. PEG-fused vs Negative Control (NC) isograft of 0.5cm length sampled 3 each at 7,21,42d PO for axonal/NMJ/muscle morphology and/or function (CAPs, CMAPs) and at least 6 each weekly and/or for 42d for behavioral function (SFI, von Frey tests). 30 Chronic Lewis rats, 15 acute donor Lewis rats. Performed one repair with PEG fusion: A: The animal had a recovery SFI score B: No self-mutilation was observed. C: No axonal morphology or VF tests were performed with this 1st batch of animals. **Aim 2B.** Baseline Data. PEG-fused vs Negative Control (NC) isografts of 1.0 cm length sampled 3 each at 7,21,42d PO for axonal/NMJ/muscle morphology and/or function (CAPs, CMAPs) and at least 6 each weekly for 42d for behavioral function (SFI, von Frey tests). 15 rats for each PEG and NC protocol. 30 chronic Lewis rats, 15 acute donor Lewis rats. Performed seven repairs with PEG fusion and three without PEG (NC): A: 3 out of 7 PEG fused animal had recovery SFI scores. B: None of the Negative controls were recovered. C: No self-mutilation was observed. D: No axonal morphology or VF tests were performed with this 1st batch of animals

Aim 2C: Environmental Augmentation data. PEG-fused vs Negative Control (NC) isograft of 1.0 cm length with local augmentation (FK506) as directed by Dr. Alderete sampled 3 each at 21,42d PO for axonal/NMJ/muscle morphology and/or function (CAPs, CMAPs) and at least 6 each weekly for 42d for behavioral function (SFI, von Frey tests). 30 chronic Lewis rats, 15 acute donor Lewis rats

Analyze and compare data from different sub-Aims. Data from 137 chronic and 45acute rats.

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.

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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TABLE OF CONTENTS

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). Additional strategies will be explored to enhance the conventional axonal repair and incorporation as well as optimizing the validity and percentage of PEG Fused axons. 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.

Re-Negotiated Combined and Approved SOW

(PEG FUSION) AUTOGRAFT RECONSTRUCTION IN LARGE ANIMAL MODEL OF SEGMENTAL NERVE INJURY (SNI)

STATEMENT OF WORK – Re-Negotiated 15 March 2022
PROPOSED START DATE 15 SEP 2022

Site 1: SAMMC
3851 Roger Brooke Drive
JBSA-Ft Sam Houston, TX 78234
PI: COL Joseph Alderete
PM: Raul Corpus

Site 2: University of Texas at Austin
Department of Neuroscience
100 E. 24th St., NHB. 2.504C7000
Austin, Texas 78712-0805
PI: George D. Bittner, PhD (GB)

Site 3: Johns Hopkins University School of Med
601 N Caroline St,
Baltimore MD 21287
PI: Jaimie T. Shores, MD

Site 4: Metis Foundation
PI: Margaux Salas, PhD

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**	**Research Sites**			
Aim 1 will require 120 rats (n=6 for Groups 1, 2 and n=18, for Groups 3-8; 3 time points).	Months	**SAMMC**	**UTA**	**JHU**	**Metis**
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	JA	GB		
Milestone # 1: Obtain IACUC approval for rat, and porcine 3	3	JA	GB		
Subtask 2: Submit documents for ACURO for rat, and porcine 2-3	2-3	JA	GB		
Subtask 3: Recruitment/training of personnel; Purchase of reagents/ equipment. 1-6	1-6	JA	GB		
Milestone # 2: Obtain ACURO approval for rat and porcine studies	6	JA	GB		

Major Task 2: Assess the effect of the PEG-fusion in rat sciatic nerve SCNI by varying established surgical technique with mechanical enhancement and environmental augmentation in order to validate a gold standard (best) method.	6-24				
Aim 1: Obtain multimodal PEG- fusion baseline data and Environmental Augmentation data on female Sprague Dawley rat sciatic, single cut, nerve model systems for behavioral recovery (SFI, von Frey tests), axonal/ NMJ/muscle morphology and function (CAPs, CMAPs).	0-12		GB		
Subtask Aim 1A: Train four surgeons from DOD RESTOR San Antonio to PEG-fuse singly cut rat sciatic nerves.\assayed by weekly SFI behavioral tests for 6 weeks. 6 rats/surgeon. **24 Sprague Dawley (SD) chronic rats.**	0-6	JA	GB		
Milestone 1. Completion of subtask 1A	6		GB		
Subtask Aim 1B: **Baseline Data.** PEG-fuse singly cut Sprague Dawley rat sciatic nerves enhanced by FK506/IGF1 application and mechanical enhancement (FSG or Fibrin Sealant Glue versus microhook nerve tape) assayed by behavioral recovery (SFI, von Frey tests), axonal/NMJ/muscle morphology and function (CAPs, CMAPs). Compare to PEG and NC historical data. **15 Sprague Dawley (SD) chronic rats.**	4-12		GB		
Milestone 2. Completion of subtask 1B	12		GB		
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. 2 groups, total 36 rats. (Table 1, group 7-8).	6-18		GB		
Subtask 5: Standard repair + PEG fusion, combined with **environmental augmentation** (FK506 or IGF1) in rat sciatic SCNI for improving nerve healing and post-procedural function. 2 groups, total 36 rats. (Table 1, group 7-8).	6-18		GB		
Subtask 6: Based on results above perform Standard Repair + PEG Fusion + Environmental Augmentation + Mechanical Augmentation. 6 groups, total 84 rats. (Table 1, Group 1-6).			GB		
Subtask 7: 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				MS

Subtask 8: Perform histopathology, IHC, nerve histomorphometry and genomic evaluations to assess qualitative and quantitative parameters of axonal regeneration and distal NMJ integrity.	6-24		GB		
Milestone # 3: Demonstrate PEG fusion in rat sciatic nerve injury (SCNI) is adaptable in mechanical strength and enhanced healing and meet criteria for stability and axonal regenerative capacity	24		GB		
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.	**Months**	**SAMMC**	**UTA**	**JHU**	**Metis**
Aim 2 will require 108 rats (n=18, 3 time points).					
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 9: 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		GB		
Subtask 10: 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		GB		
Subtask 11: 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		GB		MS
Subtask 12: Perform histopathology, IHC, nerve histomorphometry and genomic evaluations to assess qualitative and quantitative parameters of axonal regeneration and distal NMJ integrity.	12-24		GB		
Milestone # 4: Complete in vivo studies and determine whether PEG fusion of nerve autografts improve functional outcomes in Sciatic Nerve Gap repair model.	24		GB		
Aim 2: Baseline data and Environmental Augmentation data. Obtain multimodal PEG- fusion baseline data on female Lewis rat sciatic, autograft, nerve model systems for behavioral recovery (SFI, von Frey tests), axonal/NMJ/muscle morphology and function (CAPs, CMAPs).	0-24		GB		
Milestone 3. Completion of subtask 2A	16		GB		
Subtask Aim 2B. Baseline Data. PEG-fuse and Negative Control (NC) autografts of 1.0 cm length sampled 3 each at 7,21,42d PO for axonal/NMJ/muscle morphology and function (CAPs, CMAPs) and at least 6 each weekly for 42d for behavioral function (SFI, von Frey tests). 15 rats	8-22		GB		

for each PEG and NC protocol. 30 chronic Lewis rats, 15 acute donor Lewis rats total.					
Milestone 4. Completion of subtask 2B	22		GB		
Subtask Aim 2C: Environmental Augmentation data. PEG- fuse and Negative Control (NC) autografts of 1.0 cm length sampled 3 each at 21,42d PO for axonal/NMJ/muscle morphology and function (CAPs, CMAPs) and at least 6 each weekly for 42d for behavioral function (SFI, von Frey tests). 12 rats for this PEG protocol. 12 chronic Lewis rats, 6 acute donor Lewis rats total.	12-22		GB		
Milestone 5. Completion of subtask 2C	22		GB		
Analyze and compare data from different sub-Aims utilizing 111 chronic and 36 acute rats	12-24		GB		
Write manuscripts for publication, collect additional data as needed	18-24		GB		
Milestone(s) Achieved: completion of each subtask	24		GB		
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 model.	**Months**	**SAMMC**	**UTA**	**JHU**	**Metis**
Aim 3 will require 36 porcine (n=6, 6 groups)					
Major Task 4: Validate the effect of PEG fusion on functional nerve recovery in porcine forelimb median nerve injury model.	16-36				
Subtask 13: 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	JA			
Subtask 14: Assess the effect of PEG fused autograft in porcine forelimb median nerve gap injury with 4 cm gap. 6 pigs per group. (Table 1, Group 17-18)	16-36	JA			
Subtask 15: Assess the effect of PEG fusion combined with local environmental augmentation (FK506 vs IGF1) in porcine forelimb critical median nerve gap injury (4 cm) model. 2 groups, 6 pigs per group. (Table 1, Group 19-20)	16-36	JA			
Subtask 16: Assess the effect of PEG fusion combined with mechanical strength augmentation (FSG) in porcine forelimb critical median nerve gap injury (4 cm) model. 2 groups, 6 pigs per group. (Table 1, Group 19-20)	16-36	JA			
Subtask 17: Assess the effect of PEG fusion combined with mechanical augmentation and environmental augmentation for healing and strength in porcine forelimb critical median nerve gap injury (4 cm) model. 2 groups, 6 pigs per group. (Table 1, Group 19-20)		JA			

Subtask 18: Perform serial in vivo functional evaluations (Video Gait Analyses) as well as electrophysiologic studies (EMG and nerve conduction) to assess nerve regeneration.		JA			
Subtask 19: Perform histopathology, IHC, nerve histomorphometry and genomic evaluations to assess qualitative and quantitative parameters of axonal regeneration and distal NMJ integrity.	16-40	JA			
Milestone # 5: Complete in vivo studies and determine whether primary nerve repair and nerve autografts with PEG fusion improve functional outcomes in preclinical porcine model.	40	JA			
Major Task 5: Data analysis, interpretation of results, comprehensive statistical analysis of study outcomes.	36-48				
Subtask 20: Data analysis, interpretation of results, comprehensive statistical analysis of study outcomes and final project report for DOD	36-48	JA			
Subtask 21: Final manuscript preparation on entire study.	36-48	JA			
Milestone # 6: Development of a clinically relevant, optimized protocol for PEG-fusion translatable to peripheral nerve injuries following civilian or combat trauma.	48	JA			
SPECIFIC AIM 4: Translate porcine techniques developed/mastered in Aim 1-3 to demonstrate inter-institutional technical translation and to further examine techniques for mechanical augmentation including in microneedle nerve repair tape	**Months**	**SAMMC**	**UTA**	**JHU**	**Metis**
Aim 4.1: Evaluate standard PEG fusion and repair (9-0 Prolene x 4 suture ((n=3))-versus standard PEG Fusion with FSG and only 2 sutures. (n=3). Outcomes = pullapart strength.	36-40	JA			
Aim 4.2: Evaluate mechanical augmentation of PEG fusion and repair with coaptation device-Immediate standard microsurgical repair of median neurotomy with mechanical augmentation utilization a nitinol mi- croneedle “nerve tape” (nitinol and small intestine submucosa (SIS)) manufactured nerve coaptation de- vice in combination with PEG fusion (n=3) Outcomes=Pull apart strength	36-40	JA			
Aim 4.3: Compare the mechanical strength of the PEG Fusion repair with MNT versus FSG and if this strength differences translates to more reproducable PEG Fusion and ultimate function.	36-40	JA			

SPECIFIC 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.	**Months**	**SAMMC**	**UTA**	**JHU**	**Metis**
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			JS	
Subtask 2: Obtain IACUC and then ACURO approvals of protocols at JHU.	25-28			JS	
Milestone #1: Regulatory approval obtained.	28			JS	
Subtask 3: Execute Aim 5.1 (Aim 5.1**: Short term assessment** of median nerve SC neurotomy repaired with (n=1) and without (n=1) PEG fusion.) • Train and assess subjects for Kluver pinch board function and volitional grip strength testing. • 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. • Behavioral function assessments with modified Kluver pinch board and volitional grip strength testing of bilateral extremities performed. • Return to OR at day 21 for operative exploration, stimulated grip strength testing, EMG/NCS testing, and biopsy of normal muscle, muscle of median muscle group, and median nerve distal to repair site • Reconstruction of median nerve defect with autograft or primary repair. • 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.	28-37			JS	

• Observation for up to 6 months of return of function and socialization skills that would enable discharge into "retirement colony" or return to research colony at large.					
Subtask 4: Execute Aim 5.2 Aim 5.2: **Long term assessment** of median nerve neurotomy. Eight total subjects will be studied: • 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 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) Forearm median neurotomy in randomly chosen (left or right) male Rhesus Macaques with (n=1) and without (n=1) PEG fusion applied utilizing autogenous nerve graft for segmental reconstruction. EMG/NCS and stimulated grip strength testing before and after repairs performed. Sham exposure of contralateral arm with testing performed. • Histomorphometry and Electron Microscopy: Muscle and nerve biopsy specimens will be evaluated by light, infrared, and electron	29-42			JS	

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 #2: Completion of primate work and written summary of study results for presentation at investigators and initiate manuscript preparation.	42	JA		JS	

SAMMC/RESTOR Fuse Pig Numbers and Groups

#	Table 1: Experimental Design Treatment Groups (n=18)	Local Treatment	Description Evaluations at 3 timepoints (4,7,21 days) 6 rats each
1	Sham	-	**Aim:** Limit denervation atrophy in Optimized PEG fusion restoration of axonal continuity for acute reinnervation of targets These groups will serve as control groups. These groups include both negative controls and gold standard treatments of nerve injuries.
2	Nerve transection without repair	-	
3	Nerve transection with epineural repair (Standard of Care)	-	
4	Nerve transection with **PEG Fusion**	-	
5	Nerve transection with delayed **(24 hours)** epineural repair	-	
6	Nerve transection with delayed **(24 hours) PEG Fusion**	-	
7	Nerve transection with epineural repair (Standard of Care) **at 24 hours**	FK506 or NAF or MNT	
8	Nerve transection with **PEG Fusion at 24 hours**	FK506 or NAF or MNT	
9	Nerve gap (5 mm) repair with epineural ANG (Standard of Care)	-	**Aim:** Our group will expand upon preliminary work defining the parameters (graft length, timing, technique) for successful application of PEG fusion technology coupled with nerve autografts across a spectrum of animal models.
10	Nerve gap (5 mm) repair with autologous PFAR	-	
11	Nerve gap (15 mm) repair with epineural ANG (Standard of Care)	-	
12	Nerve gap (15 mm) repair with autologous PFAR	-	
13	Nerve gap (**5 or** 15 mm) repair with epineural ANG (Standard of Care)	FK506 or NAF or MNT	
14	Nerve gap (**5 or** 15 mm) repair with autologous PFAR	FK506 or NAF or MNT	
Optimizing ideal dose and delivery of NPCs and preclinical application in a porcine and primate critical nerve gap model (n=6 and n=1 or 3, respectively), followed up 30 and 100 days.			
15	Nerve transection with epineural repair (Standard of Care)	-	**Aim:** PEG fusion will be studied across a spectrum of animal models and treatment scenarios (as describe above) to improve rapid target reinnervation in PNI.
16	Nerve transection with **PEG Fusion**	-	
17	Nerve gap (40 mm) repair with epineural ANG (Standard of Care)	-	
18	Nerve gap (40 mm) repair with autologous PFAR	-	
19	Nerve gap (40 mm) repair with epineural ANG (Standard of Care)	FK506 or NAF or MNT	
20	Nerve gap (40 mm) repair with autologous PFAR	FK506 or NAF or MNT	
21	Nerve transection with epineural repair (Standard of Care) n=1		**Aim:** The groups in this task might be changed planned according to the best working group in porcine studies.
22	Nerve transection with **PEG Fusion** n=3		
23	Nerve gap (40 mm) repair with epineural ANG (Standard of Care) n=1		
24	Nerve gap (40 mm) repair with autologous PFAR n=3		
PFAR: PEG Fused Autograft Reconstruction, ANG: Autologous Nerve Graft, NAF: Neuraptive AxoFuse, MNT: Micro-Hook Nerve Tape.			

Data Analysis and Interpretation: Sections containing more than one subsample will be analyzed for their intra-sample heterogeneity. For electrophysiological parameters, unpaired two-tailed Student's t-tests will be performed to compare the mean normalized amplitudes and latencies between control and experimental groups. For gait-analysis parameters, unpaired two-tailed Student's t-tests with Levene's testing will be used to compare the normalized run parameters between groups. Between-group means will be assessed for statistically significant differences using one-way ANOVA followed by two-tailed, two-sample, independent Student's t-tests and the Bonferroni correction to adjust for multiple comparisons. For nerve histomorphometry, unpaired two-tailed Student's t-tests will be used to compare the axon counts, axon density, axon diameter, and nerve fiber diameter between experimental groups. A priori sample size and power calculations based on previous results from similar experiments are performed to detect a minimum 3-fold increase of mean axon counts, a 50% increase in mean normalized CMAP amplitude, and a 50% reduction in mean normalized CMAP latency in the NPC-treated rats at the familywise error rate of 5% and 80% power. A p-value of <0.05 will be considered to be significant in all analyses. All analyses will be performed using SAS statistical analysis software (SAS Institute, Inc., Cary, North Carolina).

JHU Rhesus Macaque Experimental Plan – Specific Aim 5			
Group	**Animal Numbers**	**Treatment Description**	**Assessment**
1	1	Short term outcome: Single median nerve cut and standard repair alone.	Pre-op: volitional grip strength and modified Kluver board pinch oppositional pinch training and assessment. Intra-op: NCS/EMG with CAP and CMAP, stimulated grip strength Post-op: Weekly volitional grip strength and modified Kluver board oppositional pinch assessment. 21 days: Operative assessment with NCS/EMG with CAP and CMAP, stimulated grip strength testing, and non-median innervated muscle and median innervated muscle biopsy and nerve biopsy 4 cm distal to repair with repair of biopsy site with or without PEG fusion Observation for up to 6 months with weekly Kluver board pinch and volitional grip strength MRN/DTI after 4 months with stimulated grip strength testing under anesthesia Exit study
2	1	Short term outcome: Single median nerve cut and standard repair + PEG fusion	
3	1	Long term outcome: single median nerve cut and standard repair alone.	Pre-op: volitional grip strength and modified Kluver board pinch oppositional pinch training and assessment. Intra-op: NCS/EMG with CAP and CMAP, stimulated grip strength Post-op: Weekly volitional grip strength and modified Kluver board oppositional pinch assessment. Observation for up to 11 months or plateau of functional outcome measures with weekly Kluver board pinch and volitional grip strength testing, MRN/DTI every 4 months with stimulated grip strength testing under anesthesia 11 months or plateau of function: Operative assessment with NCS/EMG with CAP and CMAP, stimulated grip strength testing, and non-median innervated muscle and median innervated muscle biopsy and distal forearm nerve biopsy with repair of biopsy site with or without PEG fusion
4	1	Long term outcome: Single median nerve cut and standard repair + PEG fusion	
5	1	Long term outcome: single median nerve cut and repair with FGS alone.	
6	1	Long term outcome: single median nerve cut and repair with FGS alone + PEG fusion.	

			Observation for 1 month with weekly Kluver board pinch and volitional grip strength testing. Exit study.
7	1	Long term outcome: single median nerve cut and repair with “Nerve Tape” nitinol microhook coaptation device alone	• For all groups: Muscle and nerve biopsies with undergo histomorphometric analysis and electron microscopic analysis as indicated in specific aims narrative (axon count, g-ratio’s, muscle fiber cross sectional area, NMJ junction staining, etc.)
8	1	Long term outcome: single median nerve cut and repair with “Nerve Tape” nitinol microhook coaptation device + PEG fusion	
9	1	Long term outcome: single median nerve cut and reconstruction with autogenous nerve grafting alone	
10	1	Long term outcome: single median nerve cut and reconstruction with autogenous nerve grafting + PEG fusion	
Total	**10**	**Male Rhesus Macaques (ages 2-3)**	

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 7: 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 11: 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 2 for Renegotiation Narrative) among all parties involved in specific aim 1. This resulted in the Combined Approved SOW we are now engaging. 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 single cut and autograft best practices and surgeon skill translation. Dr Cathy Yang PhD as Peg Fusion scientific advisor who is the lab manager and neuroscientist for Dr Bittner at UT Austin has proven invaluable in technical transition of surgical skill in training my surgeon team for maximumly successful Peg Fusion. **Sub-Task 1A.**

Given that PEG Fusion can require skill and understanding beyond that of research personnel; translation into the operating room has been a sticking point and critique (Brogan and Dy. ASSH Best Paper 2022. *PEG Fusion Does Not Improve Medium Term … Rat Sciatic Nerve Transection and Repair*). In sharp contranst to many labs we have undertaken an algorithmic approach to training and verification under the technology SME's Dr George Bittner and his wife Dr Cathy Yang. This husband and wife team discovered PEG Fusion 30+ years ago. 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 Dr Yang has also established the electrodiagnostic protocol that best demonstrates on-table success of a PEG Fusion repair in any organism. The groups that have not found PEG Fusion to be efficacious did not formally train under any established system nor did they verify their on table fusion success. **SubTask 1A.**

SOW Specifics:

Aim 1: Obtain multimodal PEG- fusion baseline and Environmental Augmentation data on Sprague Dawley rat sciatic, single cut, nerve model systems for behavioral recovery (SFI, von Frey tests), axonal/NMJ/muscle morphology and function (CAPs, CMAPs).

Aim 1A: Train four surgeons from DOD RESTOR San Antonio to PEG-fuse singly cut rat sciatic nerves assayed by weekly SFI behavioral tests for 6 weeks. 6 rats/surgeon. 24 Sprague Dawley (SD) or Lewis chronic rats. Two surgeons from DOD RESTOR have been trained in UT Austin and two additional surgeons also trained on April 28th 2023; 100%.

Aim 1B: Baseline Data . PEG-fuse *singly cut* Sprague Dawley or Lewis rat sciatic nerves enhanced by FK506 application assayed by behavioral recovery (SFI, von Frey tests), axonal/NMJ/muscle morphology and/or function (CAPs, CMAPs). Compare to PEG-fused and NC historical data. 15 Sprague Dawley (SD) or Lewis chronic rats. 1: Obtain von Frey test Baseline data (see attached) papers : 80%. 2: Established the tisseel application procedure with the help of the Baxter staff

3: Operated 4 SD rats with only two sutures and tisseel applications. A: Only one of the 4 operated animals recovered with an SFI score better than -69.

B: It appears that the repairs with tisseel had minimal inflammation (or neuroma) without axon sprouting outside of epineural sheaths. For the future surgeries for this Aim, we are planning to perform the traditional neurorrhaphy: at least 4 sutures without tisseel application. C: Only one of 4 animals (25%) had very minor self-mutilation: our historical data in the lab was greater than 50%, indicating that tisseel might have helped prevent from self-mutilation although the number of animals is relatively small to make a concrete conclusion

Aim 2: Baseline data and Environmental Augmentation data. Obtain multimodal PEG- fusion baseline data on Lewis rat sciatic isograft, nerve model systems for behavioral recovery (SFI, von Frey tests), axonal/NMJ/muscle morphology and/or function (CAPs, CMAPs). **Aim 2A.** Baseline Data. PEG-fused vs Negative Control (NC) isograft of 0.5cm length sampled 3 each at 7,21,42d PO for axonal/NMJ/muscle morphology and/or function (CAPs, CMAPs) and at least 6 each weekly and/or for 42d for behavioral function (SFI, von Frey tests). 30 Chronic Lewis rats, 15 acute donor Lewis rats. Performed one repair with PEG fusion: A: The animal had a recovery SFI score B: No self-mutilation was observed. C: No axonal morphology or VF tests were performed with this 1st batch of animals. **Aim 2B.** Baseline Data. PEG-fused vs Negative Control (NC) isografts of 1.0 cm length sampled 3 each at 7,21,42d PO for axonal/NMJ/muscle morphology and/or function (CAPs, CMAPs) and at least 6 each weekly for 42d for behavioral function (SFI, von Frey tests). 15 rats for each PEG and NC protocol. 30 chronic Lewis rats, 15 acute donor Lewis rats. Performed seven repairs with PEG fusion and three without PEG (NC): A: 3 out of 7 PEG fused animal had recovery SFI scores. B: None of the Negative controls were recovered. C: No self-mutilation was observed. D: No axonal morphology or VF tests were performed with this 1st batch of animals
Aim 2C: Environmental Augmentation data. PEG-fused vs Negative Control (NC) isograft of 1.0 cm length with local augmentation (FK506) as directed by Dr. Alderete sampled 3 each at 21,42d PO for axonal/NMJ/muscle morphology and/or function (CAPs, CMAPs) and at least 6 each weekly for 42d for behavioral function (SFI, von Frey tests). 30 chronic Lewis rats, 15 acute donor Lewis rats

Analyze and compare data from different sub-Aims. Data from 137 chronic and 45acute rats.

Specific Aim 3:

We elucidated the most reproduceable and fastest surgical approach to the porcine forelimb single cut and segmental gap reconstruction through PRORPFY19 to be an ulnar to median nerve autograft. **(Alderete et al 22);** however, this became the source of multiple revisions to our AFIRM III IACUC protocol for animal safety and utility.In denervating the ulna- sided sensation before the median nerve innervation completely back on line the animals were though to be at risk for hoof ulceration. We therefore adapted an ulnar sided neo-orthotic that the pigs did not chew off which we tested on our limb-transplant training animals. This adaptation satisfied our vets and allowed the final IACUC OCT 23 through contract to UT Health San Antonio as surrogate lab to RESTOR/59th..

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 experiments OCT 23 which was approved at UT Health Science Center San Antonio through contract to Metis/RESTORE/59th MDW and ACURO pending. (See Appendix 4.)**

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

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 submitted JNS23-2222**
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 presented an abstract at 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."

I hope to find in our next reporting period that we have the best properties for our Fibrin Clot carrier, Tisseel, with the optimal concentration of Tacrolimus. We should at the least be able to demonstrate that the addition of FSG improved or did not improve PEG Fusion neurorrhaphy in rat and pig single cuts. We should be just starting the pig autograft work with the rat autograft work well under way.

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.

Impact (Cont'd):

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 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 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 we will have completed rodent and porcine experiments in the AY '23/24 period with NHP experiments starting in Jan 2024.

I retired 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 or Co-Investigator to spur animal work to completion through my lab at UT Health Science Center San Antonio

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.

The differences between MIPR, Intramural funds, and extramural funds had us go to UT Health Science Center San Antonio for throughput and animal expenditure without expending funds too aggressively.

Finally, MIPR funds between BAMC and CIRS/59th can often become "misplaced," taking a great deal of time finding funds. None have been lost in this experience but precious time spent.

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

.

Significant changes in use or care of human subjects

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*)

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.

Tyler A. Smith, Cameron L. Ghergherehchi, Kelly C.S. Roballo, Michelle Mikesh, Haley O. Tucker, **Jaime T Shores, Joseph Alderete, Erik K. Weitzel,** Jared A. Bushman, **George D. Bittner.** 2022. Immunotolerance of polyethylene glycol-fused sciatic allografts from Brown-Norway rats into Lewis host rats. Frontiers in Cellular Neuroscience. December 2021 expected Submission

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 presented at 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. 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

Project Role: Lab Manager Researcher Identifier (e.g. ORCID ID): RESTOR 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

Researcher Identifier (e.g. ORCID ID): 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

Researcher Identifier (e.g. ORCID ID): Nearest person month worked: 3.0

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: Monzer Alatrach

Project Role: URA, UTA

Researcher Identifier (e.g. ORCID ID): Nearest person month worked: 0.8

Contribution to Project: Ex vivo survival of sciatic nerve allografts

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: PRORP FY 19 Guidance Work Results Paving Way

Appendix 2: AFIRM III Renegotiation Documents

Appendix 3: FSG and FK506 Assisted PEG Fusion

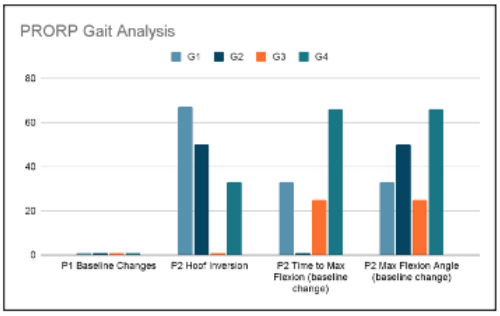
Appendix 4: UTA IACUC and ACURO

Appendix 1: Y2Q2

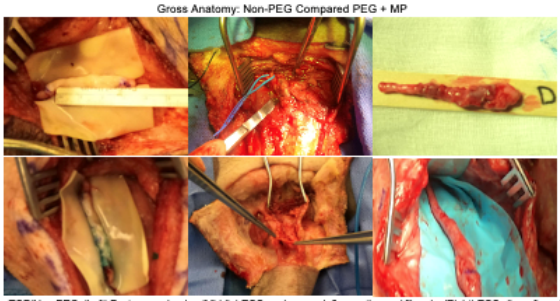
PRORP FY 19 Success Informing and Streamlining AFIRM III Studies

Gait Analysis

Gait Summary	
GM1: • P1 baseline changes: insignificant • P2 baseline changes: ○ Hoof inversion: ⅗ or 66% ○ Time to max flexion: ⅓ or 33% ○ Max angle: ⅓ or 33%	GM2: • P1 baseline changes: insignificant • P2 baseline changes: ○ Hoof inversion: ½ or 50% ○ Time to max flexion: 0% ○ Max angle: ½ or 50%
GM3: • P1 baseline changes: insignificant • P2 baseline changes: ○ Hoof inversion: 0% ○ Time to max flexion: ¼ or 25% ○ Max angle: ¼ or 25%	GM4: • P1 baseline changes: insignificant • P2 baseline changes: ○ Hoof inversion: ⅓ or 33% ○ Time to max flexion: ⅗ or 66% ○ Max angle: ⅗ or 66%



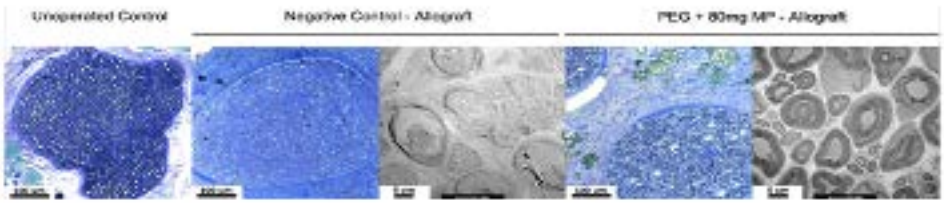
Gross Anatomy: Non-PEG Compared PEG + MP



TOP/Non-PEG: (Left) Post neurorrhaphy; (Middle) EOS mod-severe inflammation and fibrosis; (Right) EOS allograft mod-severe inflammatory/hemorrhagic injury with undistinguishable nerve-fibrotic layers. BOTTOM/PEG+MP: (Left) Post neurorrhaphy; (Middle) EOS mild inflammation and fibrosis; (Right) EOS allograft demonstrating mild inflammation with distinguishable separation between nerve-fibrotic layers.

POD#13: Non-PEG Compared PEG Phase 2 Gait Abnormalities

(Left) Pig treated without PEG demonstrating moderate-large medial hoof inversion. (Right) Pig treated with PEG (-MP) demonstrating mild medial hoof inversion (those treated with +MP had no deviation).



Appendix Y2Q1: Original Renegotiation Narrative Prior to METIS Personnel Sub

SAMMC and 59th MDW AFIRM III Sub-award Renegotiation Scientific Justification

W81XWH2020029/"Multimodal Approach to Improve Functional Recovery Following Acute and Delayed Peripheral Nerve Injury Repair"
PI: COL Joseph F Alderete MD

Background

PEG Fusion (Poly-ethylene glycol fusion) is the process of cellularly fusing nerve after nerve injury. Most of the nerve injuries from combat 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. Finally, our original design was to make the final jump into the most promising science of PEG Fusion in allograft, which shows the greatest combat nerve injury potential.

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.

Historical Creation of the Team

Our initial collaboration started with (COL Joseph Alderete MD) SAMMC/RESTOR, Dr George Bittner PhD (creator of PEG Fusion at UT Austin) and Dr Jaimie Shores MD (Microvascular Nerve Surgeon at Johns Hopkins University). Each of these team PI's have many members of their inherent teams of which you see Col Erik Weitzel MD (59th MDW/RESTOR Chair), MAJ Julie Nuelle MD, MAJ Benjamin Plucknette MD (SAMMC Hand and Micro Nerve team), and Dr Vijay Gorantla MD (Consultant from Wake Forest, WFIRM) as part of the RESTOR team.

Historical Team Objectives for Original AFIRM III submission

- Johns Hopkins University: Site P1. Dr Jaimie Shores MD. Originating site of the human clinical trial and Non-human primate work. I wanted Dr Shores to do the NHP with me present but at JHU because of his work with monkey median and ulnar nerve in upper limb allotransplantation.

- UT Austin: Site P2. Dr George Bittner. Dr Bittner has studied PEG Fusion in nerves for over 30 years. He is the father of the technology. There is significant work, among this collaborative effort, showing excellent potential in single cut, autograft, and allograft rodent work and that the PEG Fusion immediate environment may be sealed off from the immune system (Smith,

Bittner, Shores, Alderete, Weitzel NRR-D-20-00943 2021). Dr Bittner was yoked to train the SAMMC surgeon team and house the initial autograft work and all the final translation to allograft. *As part of AFIRM III he was not funded.*

- SAMMC RESTOR: Site P3. COL Joseph F Alderete MD. My charge was to organize the SAMMC surgeon team, designing its education in PEG Fusion around rodent single cut and autograft PEG Fusion. I would then manipulate the PEG Fusion success rate and the successful healing of non-PEG Fused axons by using fibrin sealant glue, FK506 (potent immunomodulator) and microhook nerve tape (make surgery less fussy) on rodents at RESTOR then translating the lessons learned to pigs for final adaptation into NHP and humans for P1 Clinical Trial. I was also one of the 2 sites selected to manipulate the human clinical trial as part of site P1.

- Virginia Commonwealth University: Site P4. Jonathan Isaacs MD. Facilitated the Microhook nerve tape and a separate study looking at Anterior horn stem cells to augment or in place of PEG Fusion for potential nerve repair. *He was not funded as part of AFIRM III.*

Evolution of the current SOW

A. Once we were notified of the original award, we were confronted with 2 immediate problems. We chose to apply for AFIRM III as a consortium of separate PI; however, we had a great deal of synergistic work not knowing how much of this work was highly interdependent. Sites P2 and P4 were not funded. We did not know initially, until award negotiations, how problematic this would be. The San Antonio Military Medical Center/RESTOR AFIRM application assumed funding UTA 1.8M application for data to be confirmed at San Antonio. That confirmation I saw as an important step in getting this surgical technique out of the lab and to the warfighter.

B. Our group would also expand upon preliminary work defining the parameters (graft length, timing, technique) for successful application of PEG fusion technology coupled with nerve autografts across a spectrum of animal models. We would finally seek to establish strategies to improve axon re-generation across the repair site will be addressed by our group (improved coaptation alignment with Nerve Tape and or fibrin sealant glue in rodent sciatic nerves, pig median nerves, and non-human primate median nerves.

C. We also anticipate no problems completing Aim 1 for small-diameter axons because we and others have already successfully PEG-fused axons of similar diameter rats, rabbits, guinea pigs and now humans. The original we were UTA and Vanderbilt/Trevino a very important point to surface later….

Course of Action:
Given that we were going to have to adapt the study through our funded sites to maintain all collaborations we sought initially to house all animal studies through SAMMC RESTOR and the Human Clinical Trial through Johns Hopkins. Because of the way the grant was structured in application the non-human primate work would be allocated to me but immediate sub to Dr Shores at JHU because we were under the impression a team would not be funded to do animal work along with application to human trial. Nonetheless, I still wanted to take advantage of the JHU expertise in primate surgery rather than have Dr Shores come to San Antonio and operate on primates with an inexperienced system possible hindering result. We were thrown a couple of curves requiring flight course corrections while I was deployed this summer:

1) HJF could not originally negotiate budget in the presence of Mr. Raul Corpus, RESTOR Program Manager, because he is a contractor until NDA was in place.
2) We had to accept a certain amount of funding in 2020 by OCT because of in/out year ramifications. This was where we elected to make HJF prime for all with RESTOR 59th MW receipt for all P3 given that SAMMC does not have a great track record for fund acceptance.
3) We had to dig hard into the budget initially because Mr. Okagaki of HJF informed us that we had unanticipated costs in HJF indirect.

Thus, we negotiated among the partners to remove 4 of the Non-Human Primate from the P3 budget and I took out most of my salary for study help. A critical period developed however where I was 8 hours ahead in

Baghdad and busy with COVID patients and Combat Trauma. A member of our RESTOR team with HJF re-formatted the SOW assuming some of the responsibility of the initial rodent work and some of the non-human primate we had to negotiate away onto our RESTOR/WFIRM Wake Forest relationship in thinking it in RESTOR/SAMMCs best interest to use WFIRM as "SAMMC North" given their rich animal lab history thinking I could travel to Wake Forest to do initial rodent work leaving me to concentrate on very important porcine model manipulations feeding eventual best practices surgical technique and biologic manipulations. Hence, Wake Forest entry onto the scene as part of RESTOR/WFIRM historical partnership and consultancy. This SOW and budget justification went forward and made initial sense while I was frequently out of communication when Baghdad was getting rocketed this summer or taking coalition casualties for the final 2 weeks of budget negotiations

Where We Are Today

On 29 Aug I was notified our negotiations had been approved and we started to look at our combined Statements of Work to solidify effort for the HJF post-award team. I then noticed over a few weeks while returning home from Iraq in early October that the study had changed its shape a bit.

Alderete Budget
Hopkins:
Wake Forest
Metis Foundation:

Total Animal Budget for SAMMC/ RESTOR (direct)

Why We Need to "Re-Renegotiate."

My original intent with this work was to validate initial results obtained by my new mentor and translate them both among models and institutions. This was the premise by which our partner WFIRM got involved. However, I began to understand that this technique of PEG Fusion deserved massive respect consummate to its potential. The environment has to be just right to maximize the available axons and minimize scar/inflammatory burden of repair and wound. The idea of training on a few animals with Dr Bittner and expecting to immediately adopt expertise and technique success struck me as the usual height of arrogance in the typical surgeon mentality of "see one, do one, teach one…" This was a technique that required tutelage under an experienced mentor in both biology and technique to maximize result. Furthermore, as the original consortium began to work together a great deal on the biological ramifications, we all became convinced that keeping the science uniform ensuring useable data and teachable technique was our primary concern. We wanted to succeed or fail in PEG Fusion translation but we didn't want the technology to die on the vine because of poorly adapted work or good people trying to adapt the technique too early. Therefore, I am electing to re-structure the Sub-awards in favor of better, more responsible scientific advance and adaptation. **We will divide up the sub-award into the following:**

Goals:

1) To bring UT Austin/Dr Bittner back into the fold returning all of the rodent work to where it was created. Rationale is that if the surgical team can learn under the historical creators their biologic and technique transference would have the highest fidelity and hopefully the greatest success. No one is more likely to succeed in educating surgeons on the science and skills than Dr Bittner in PEG Fusion and I believe this is a very important consideration in bring this technique eventually to combat surgery. Finally, as you can see above the scientific ladder demands that UT be afforded the opportunity to adapt size/technique and environment in autograft nerve gaps before simply trying on larger models, especially human, because there are a number of questions that we can answer very quickly at UTA under our mentorship by Dr Bittner's team in "best practice;" whereas RESTOR would spend months re-inventing the wheel either doing the rodent work ourselves in SA or sub-contracting to WFIRM risking heterogeneous results despite excellent expertise. We truly just don't know enough about this incredible biology and there should be one established "Camelot" who can write the text on this technique rather than push out chapters of experience with no cohesion.
2) To facilitate Dr Shores performing 10 Non-Human Primate instead of 6 to achieve statistical significance/surgical equipoise in our final technique manipulations in what we have learned from surgeon training, environmental/biologic adaptation, and model translation.

3) Continue to look to Dr Gorantla and WFU/WFIRM in objective confirmatory nerve studies. Historically, the most capable histomorphology assessments of post PEG Fusion neurobiology has been UT Austin. Scientifically, this can contribute to bias. As we move onto a larger stage where the FDA and multiple university/DOD surgical teams would have to "buy in" to this technique before wide spread adoption, we will need separate assessments in microscopic "success/failure." RESOR/59th MW has a history of successful relationship with Dr Vijay Gorantla and WFIRM as does DOD/WFIRM. We will take advantage of Dr Gorantla's extensive understanding of nerve biology at the cellular level as well as involvement in DOD nerve repair alternative research to provide a very important look into the histologic analysis of the nerve downstream from the PEG Fusion repair in order to give the team measures for success and strategies to:
    a. Maximize percentage of PEG Fused axons
    b. Maximize potential of non-PEG Fused axons to organized normal biologic healing.

    This assessment has to take place at both a cellular and EMG/Functional level and will be best suited at an objective lab away from index animal work. Additionally, this has the added benefit of collaboratively facilitating WFU/WFIRM understanding of successful cellular PEG Fusion which is very difficult science and all can benefit from the cross-pollination with the experts at UT Austin.

4) To re-capture some of the administrate money for help I gave up given the magnitude of the work we will be doing and my direct experimental involvement (I want to be doing much of the surgeries with my fellowship trained partners so there is no available critique in the event of failure that "a tech or resident was doing the nerve surgery so the results are invalid.") thus we will need some admin help more than I negotiated away to keep us in the science.

How are we going to get there?

Over the last 8 months the entire team and sub-contractors have been meeting to "get to yes," with the that was awarded to WFIRM. Dr Gorantla accepted my rationale and supported a plan in the interest of RESTOR partnership that we would divide into 2 chunks.

1) The Direct Money: Approximatelywhich would be re-negotiated to bring the rodent SOW under UT again and give Dr Shores his full 10 NHP
2) The Indirect Money: Which would be awarded to WFIRM under a Vendor Services Agreement to minimize cost and overhead while helping us achieve independently validated results in immunohistochemistry, electron microscopy, and genetic studies establishing the true success or failure of the technique and guiding the "why's."

This adaptation requires that all parties re-look at their budget justifications and break out consumables from animals/salaries. These consumables are going to be re-enumerated to the HJF budget (i.e., surgical equipment can be drop shipped) to offset the overhead differences between the 2 universities and HJF; thereby maximizing the science for the government's investments.

Right Now:

1) We have acquired Dr Cathy Yang as a consultant. She is a brilliant nerve biologist and a member of Dr Bittner's scientific lab to be our consultant and bridge between the 2 TX systems so as to ensure proper surgeon training and uniform technique as well as regulatory document preparation. We are adding Capt. Casey Sabbag MD, Capt Dawn Rask MD (Ortho Resident) and LTC David Wilson MD to our Microvascular Nerve Reconstruction team who will be added to grant and IRB protocols. We will begin PEG Fusion training with Dr Yang in late March as UT Austin clears us for public gathering in lab for surgical education.

2) Dr's Shores, Bittner, and Gorantla have prepared budget break outs for Mr. Corpus and myself. They are included with SOW in final packet with accompanying budget justifications.

    a. George Bittner PhD. UT Austin.
    b. Vijay Gorantla MD. Wake Forest University/WFIRM . This was eventually denied and re-allocated to METIS for Personnel.
    c. Jaimie Shores MD. Johns Hopkins University. Total Renegotiation Awarded . Delta (plus up)

3) We have found the "hiccup" in the MIPR Budget which I believe caused all groups a great deal of confusion, most evidently myself. In the JPC 8 to 6 transition the MIPR'd amount to SAMMC/59th appears to only amount to that put into the approved cycle.

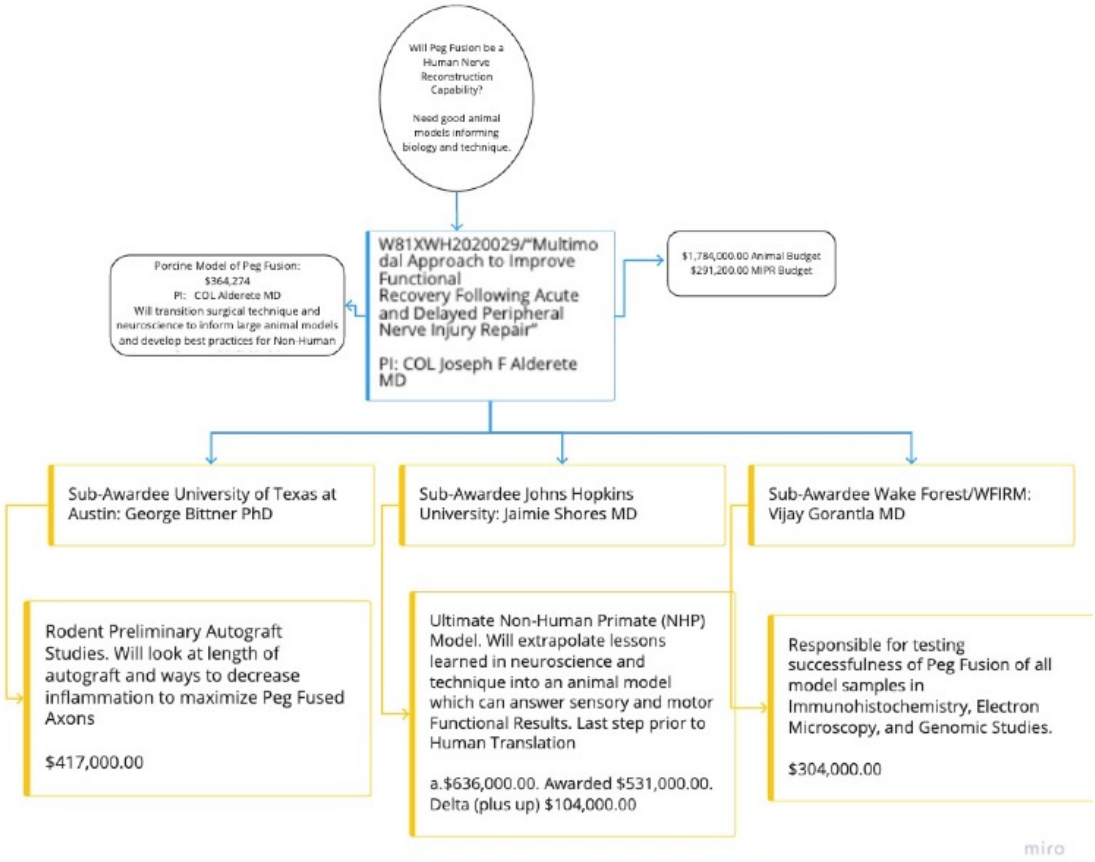
The payment schedule currently shows in FY21 Funds, in FY22 Funds and in FY23 Funds. These funds are separate from the award document for the proposal which is negotiated by USAMRAA.

**We would like to request all of these MIPR funds into RESTOR/59th MDW per attached Military Budget.

I am committed to getting this renegotiation through while maintaining all of the team relationships because of our dependence upon each other in trust and best interest. I truly believe we have a special team and stand the best chance of any in bringing this extremely important technique to battlefield medicine.

Most Respectfully,

Joseph F. Alderete Jr MD
Chief, Orthopaedic Oncology
Adult Trauma, Reconstruction, and Oncologic Surgeon
Surgical Director, Center for the Intrepid.
Associate Professor, Baylor College of Medicine
San Antonio Military Medical Center



Appendix 3: Tacrolimus Assisted PEG Fusion

FSG Assisted PEG Fusion

A fibrin sealant glue (FSG) is indicated for use as an adjunct to hemostasis in adult and pediatric patients (>1 month of age) undergoing surgery when control of bleeding by conventional surgical techniques (such as suture, ligature, and cautery) is ineffective or impractical as in the base of a nerve or to avoid damaging epineurium.. Fibrin Sealant Glues are indicated as an adjunct to standard surgical techniques (such as suture and ligature) to prevent leakage from vascular or nerve anastomoses or reconstruction of spinal dura. Nerve glue is an attractive alternative to sutures to improve the results of nerve repair. Improved axon alignment, reduced scar and inflammation, greater and faster reinnervation, and better functional results have been reported with the use of nerve glue. The current formulations of fibrin glue are routinely used for nerve grafts and transfers and may have a role in augmenting suture repairs.(Tse et al 2012) The FSG kit we use is (Freeze-Dried) is supplied as 2 mL, 4 mL and 10 mL (total volume) pack sizes with and without a proprietary dual chamber application system. In the context of FSG Assisted PEG Fusion the hemostasis component as well as the increased tensile strength are attractive; however, the glue cannot be allowed to pass into the anastomosis as it would seal the nerve prior to fusion. Thus, in FSG assisted PEG Fusion the surgeon would thaw the mixture to room temperature and keep warm until intended use. The neurrhaphy site is irrigated and hemostasis achieved. A 2ml bed of FSG is placed on the backside of the wound bed where neurrhaphy intended. One 8-0 or 10-0 Proline stitch is palced centrally, and at the most superior and inferior aspects respectively. The nerve is inlay into the FSG bed. A final central stitch is placed into the ventral or frontside of the neurrhaphy. The PEG Fusion steps are then undertaken. Finally, front side anastomosis with FSG is applied and when stable irrigated. Once repair construct is stable CAP and CMAP applied to verify PEG Fusion.

FK506 Assisted PEG Fusion

Despite substantial improvement in microsurgical techniques for nerve repair, recovery after peripheral nerve injury is usually incomplete secondary to sequella of the bodies' own inflammatory healing mechanisms. FK506, an FDA approved immunosuppressant,
improes functional recovery and reinnervation following peripheral nerve injury in animal models. There is proven efficacy of systemic fk506 at 1mg/kg/day. This dose is sub-immunosuppressant in all animal species tested including human. Sub-immunosuppressive doses are still potent neurotrophic; however, there is still concern in the dehydrated combat patient or under-resuscitated civilian trauma. Therefore, local delivery has been explored with promising results. There are papers that have used a collagen sponges as a carrier; however, they are broken down by hydrolytic reaction can confound results secondary to seroma. Their persistence is favorable even though there is no good data on the elution effects of FK506 in its local neurotrophic role. The best agent is an inflammatory neutral process that elutes over time in steady concentrations facilitating nerve regeneration over 4 weeks which would be where the PEG Fusion gradiant seems to plateau. Depending on the pharmacodynamics most antibiotics/local chemo are clinically insignificant locally at about 72 hours, greatest effect in the first 8. Our hypothesis is that the most helpful neuromodulation would be in stunting the fibroblastic recruitment and promote neurotrophic factor increase within the first 24 hours. Nonetheless, because of its favorable chemical properties an FK506 using fibrin gel as a drug reservoir that could be placed at a site of nerve injury. FK506 was incorporated into fibrin gel in solubilized, particulated, and poly(lactic-co-glycolic) acid (PLGA) microspheres-encapsulated form by colleagues at the University of Toronto (Borscher et al 2015). Its long term effect over neurotropism is not well understood. Therefore, our practice is to use a concentration of FK506 in 7mg/ml to a dose of 28mg in either a solution of 4cc of saline or 4cc of a generic created fibrin hydrogen. This was a concentration studied and efficacious through our University of Toronto colleagues. (Borscher et al 2015). If the fibrin gel application is chosen the FK506 laden gel is applied to the wound bed, much as technique described above, standard neurrhaphy performed, and then the PEG Fusion procedure is performed. Following verification of conduction the second layer of FK506 gel applied over the repair construct and the wound is closed in layers. A 1-2cc of same concentration FK506 injected into the wound bed outer layers upon closure. If the solution is chosen then

the steps are the same as above, with the exception that the deep wound be is injected directly at the neurrhaphy with 0.5ml FK506 solution, the standard suture neurrhaphy is performed in all quadrants and PEG Fusion procedure performed. Once conduction verified with CAP and CMAP the wound is bathed with 1-2 cc FK506. Wound is closed in layers around the neurrhaphy with 1cc saved to inject in progressive closure.

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APPENDIX 4: UT IACUC and ACURO for GB Work

Protocol ID: Principal Investigator(s): Protocol Title: Protocol Approved Until:

AUP-2022-00205 - AMENDMENT (1)

George Bittner, Liwen Zhou

Rapid repair of severed sciatic and spinal axons.

02/24/2023

The protocol ID listed above was approved on 09/08/2022

The University of Texas at Austin Institutional Animal Care and Use Committee
NOTICE OF APPROVAL

AAALAC Accredited Since October 29, 2001
PHS Animal Welfare Assurance Number D16-00592 (A4107-01) U.S. Department of Agriculture Registration Number 74-R-0029

The Animal Welfare Act and the Public Health Service Policy on the Humane Care and Use of Laboratory Animals require that the Institutional Animal Care and Use Committee review all use of vertebrate animals. This Animal Utilization Proposal (AUP, or protocol) must be reviewed and updated annually.

ANY change(s) to the approved protocol (i.e., personnel, procedure(s), funding source(s), etc.) must be submitted to and approved by the IACUC prior to being implemented. Any vertebrate animal expenses must be paid according to university policy and procedures.

This Approval DOES NOT VERIFY congruence with any grant funding source. Please contact the IACUC to request verification of congruence for grant award submissions.

The Principal Investigator is responsible for obtaining applicable approvals from other departments or compliance committees such as the Office of Environmental Health and Safety, Institutional Biosafety Committee, Radiation Safety Committee, etc. prior to initiating that portion of the IACUC-approved work.

Please contact the Office of Research Support and Compliance with any questions, comments, or concerns. voice: (512) 471-8871 | web: https://research.utexas.edu/ors/animal-research/ | email: IACUC@austin.utexas.edu

Protocol number:

20230056AR

Protocol title:

A novel nerve surgery technique to rapidly and permanent repair peripheral nerve injuries (PNIs) that consist of single cuts or loss of an entire segment (ablation PNIs)

Sponsor's Name	Grant Title	Proposal ID
OTHER	Multimodal Approach to Improve Functional Recovery Following Acute and Delayed Peripheral Nerve Injury Repair	PI-MANAGED-819

Dear Joseph Alderete,

Your submission has been reviewed by the OIACUC and approved on 2023-09-20. Your protocol expiration date is 2026-09-20.

This notice serves as your official OIACUC approval communication.

All approved protocol documentation including grant source information related to this submission or protocol will be available for your view from your ORCA dashboard.

This Institution has an Animal Welfare Assurance on file with the NIH Office of Laboratory Animal Welfare. The Assurance Number is A3345-01. The care and use of animals is in accordance with the NRC Publication, as revised in 2011, "Guide for the Care and Use of Laboratory Animals," and other applicable Federal regulations.

Animal Research Compliance (ARC) Program
UT Health San Antonio
7703 Floyd Curl Drive
Research Administration Building
MSC 7822
San Antonio, TX 78229
Email: ARC@uthscsa.edu
Phone: 210-567-8260

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DEPARTMENT OF THE ARMY
HEADQUARTERS, U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
810 SCHREIDER STREET
FORT DETRICK, MD 21702-5000
October 27, 2022

Director, Office of Human and Animal Research Oversight
Animal Care and Use Review Office (ACURO)

Subject: Approval of Proposal Number OR180077, Award Number W81XWH-19-2-0054 entitled, "Immediate Repair with Accelerated Recovery from Peripheral Nerve Injury Using PEG-Fusion Technologies"

Dr. George Bittner
University of Texas at Austin
Austin, TX, US

Dear Dr. George Bittner:

Reference: (a) DOD Instruction 3216.01, "Use of Animals in DOD Conducted and Supported Research and Training"
(b) US Army Regulation 40-33, "The Care and Use of Laboratory Animals in DOD Programs"

In accordance with the above references, ACURO protocol OR180077.e001 entitled, "Rapid repair of severed sciatic and spinal axons.," IACUC protocol number AUP-2022-00205, Protocol Principal Investigator Dr. George Bittner, is approved by ACURO as of 10/26/2022 for the use of rats and will remain so until modification, expiration or cancellation. This protocol was approved by the University of Texas at Austin IACUC on 08/25/2022; IACUC approval expires 02/24/2023.

Required Actions:

A. Submit to ACURO for review and approval prior to implementing:

- IACUC-approved de novo reviews of the protocol
- IACUC-approved significant changes to this protocol (see guidance document)

B. Notify ACURO within 5 business days of any of the following:

- Any noncompliance, suspensions or adverse events (see guidance document)
- Receipt of notification that the institution is under investigation by USDA
- AAALAC, International accreditation status change

For further assistance, please contact ACURO at (301) 619-6694, FAX (301) 619-4165, or via e-mail: usarmy.detrick.medcom-usamrmc.other.acuro@health.mil.

NOTE: Do not construe this correspondence as approval for any contract funding. Only the Contracting Officer or Grant Officer can authorize expenditure of funds. It is recommended that you contact the appropriate Contract Specialist or Contracting Officer regarding the expenditure of funds for your project.

Sincerely,

Krinon Moccia, DVM, MPH, DACLAM
LTC, VC, USA
Director, Animal Care and Use
Review Office

Copies Furnished:
Dr. S. John Mihic
Dr. Glen Otto
Mrs. Elena Mota
Dr. George Bittner
UT Austin IACUC
Dr. Miriam Redington
Margaret A. Hoard