Award Number: W81XWH-21-1-0441

TITLE: Nerve Repair with Polyethylene Glycol to Promote Rapid Return of Nerve Function

PRINCIPAL INVESTIGATOR: Wesley Thayer, MD PhD

CONTRACTING ORGANIZATION: Vanderbilt University Medical Center (VUMC)
Nashville, TN

REPORT DATE: July 2022

TYPE OF REPORT: Annual

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

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
July 2022	Annual	01Jul2021-30Jun2022

4. TITLE AND SUBTITLE

Nerve Repair with Polyethylene Glycol to Promote Rapid Return of Nerve Function

5a. CONTRACT NUMBER
W81XWH-21-1-0441

5b. GRANT NUMBER
OR200053

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Wesley Thayer, MD/PhD

E-Mail: Wesley.thayer@vumc.org

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

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

Vanderbilt University Medical Center (VUMC)
3319 West End Avenue, Ste 970
Nashville, TN 37203-6856

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

During the first year of the study, we have found that our institution was able to successfully deploy this study in the clinical setting, including approval of the study protocol by governing bodies, reliable referral of patients for enrollment, acquisition of required supplies and study drugs, implementation of the study protocol into the intraoperative workflow, reliable follow-up of patients for postoperative study assessments, and safety of the study drug.

15. SUBJECT TERMS
PEG; Polyethylene glycol; Nerve; Transection; Injury

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRDC
U	U	U	UU	14	19b. TELEPHONE NUMBER *(include area code)*

Table of Contents

INTRODUCTION

Current strategies for peripheral nerve repair are severely limited. Techniques such as nerve grafts, tissue matrices, and nerve growth guides have been designed to enhance the number of regenerating axons.[1] Unfortunately, even with such advanced techniques, it can take months for regenerating axons to reach denervated target tissues when injuries are proximally located.[1-4] This inability to rapidly restore the loss of function after axonal injury continues to produce poor clinical outcomes.[5] Moreover, amputation rather than repair or replantation remains a widely accepted indication in circumstances of either mutilating lower extremity injuries or revascularized upper arm injuries with segmental nerve loss. [6]

BODY

Several groups have been able to drive axonal fusion of the severed cell membranes using hydrophilic polymers and have induced both morphological and functional neuronal continuity.[7-12] The reconnection of severed nerve axons using polyethylene glycol (PEG) was first performed in 1986 with the medial giant axons (MGA) of *Procambium clarkii*.[13] Other agents have also been used to induce the fusion of severed axonal ends in mammals; closely apposed ends of severed earthworm MGAs have been fused by laser beams [14] and electric fields [15]. A recent report has shown that axonal fusion is a natural mechanism utilized by primitive species to promote rapid neuronal recovery.[16] When methylene blue (MB) is used prior to the application of PEG, the percentage of fused axons appears to increase leading to rapid functional recovery of transected nerves in animal models.[7-8] Remarkably, when coupled with microsutures, we have demonstrated that PEG fusion can promote early repair with functional recovery after complete transection of peripheral nerve. All the above-mentioned repairs require that the fusion of axonal membranes take place before the deleterious process of Wallerian degeneration occurs.

The mechanisms that govern the success of a given axonal fusion technique are based on multiple factors, including benefits afforded by PEG. PEG is a hydrophilic-compound that enhances the fusion of axons in either severed or crushed settings, restoring the injured nerve's ability to generate compound action potentials across the site of injury. For decades, scientists have used PEG to fuse together cells in order to immortalize desired cell lines such as monoclonal antibody producing B-cells.[17] PEG facilitates lipid bilayer fusion by removing hydration from membrane bound proteins at or near the damage site, decreasing the activation energy required for plasmalemmal leaflets to fuse.[18-21] These same properties of PEG have been used to restore functional transmission between the proximal cell bodies and the distal severed axons after nerve injury in multiple settings.[9-12 22] Based on these longstanding reports of PEG as a fusogenic agent *in vitro* and *in vivo,* and our own experience with PEG and MB, we designed the present pilot study to evaluate the fusogenic potential of PEG after acute traumatic nerve injury in humans.

Objectives/Specific Aims/Hypothesis: PEG fusion of human sensory nerve injuries at the site of epineural repair will result in improved return of physiologic function as compared to controls without PEG. Also, for mixed nerves containing both motor and sensory axons with segmental defects up to 3 cm, PEG delivery at both autograft repair coaptation sites will restore early safe function. These repairs must be performed within 72h after injury before Wallerian degeneration prevents rescue of the distal axons. We also hypothesize that PEG safety profile will be within acceptable limits for early clinical implementation and allow for expansion to multicenter trials.

1. **Specific Aim 1:** Determine if coaptation site delivery of PEG is superior to repair alone for lacerated nerves requiring simple epineural repair in human upper extremity sensory nerve injuries.

2. **Specific Aim 2:** Determine if dual coaptation site delivery of PEG with autografts is superior to autograft alone for segmental defects in human upper extremity injuries.

What was accomplished under these goals?

Specific Aim 1: Determine if coaptation site delivery of PEG is superior to repair alone for lacerated nerves requiring simple epineural repair in human upper extremity sensory nerve injuries.	**Timeline**	**Completed/ In Progress/ Not Initiated**
	Months	
Major Task 1: Approvals from regulatory agencies.		
Subtask 1: Prepare Regulatory Documents and Research Protocol for Study		
Review IRB documents and confirm approval status	1	Completed
Submit any changes to VUMC Central IRB review	1	Completed
Submit for USAMRDC review (ORP/HRPO)	1	Completed
Review IND documents and confirm approval status	1	Completed
Clinicaltrial.gov registration	1	Completed
Submit amendments, adverse events and protocol deviations as needed	As Needed	Completed
Coordinate with IRB for annual continuing review	Annually	Completed
Milestone Achieved: Local IRB approval through VUMC	1	Completed
Milestone Achieved: HRPO approval for all protocols	1	Completed
Major Task 2: Coordinate Study Staff for Clinical Trials		
Subtask1: Hiring and Training of Study Staff		
Advertise and interview for TBD project related staff	1-3	Completed
Coordinate hiring and training TBD staff	1-3	Completed
Milestone Achieved: Research staff trained	1-4	Completed
Major Task 3: Participant Recruitment, Intervention, Follow-up/Participant Evaluation		
Subtask 1: Recruitment		In Progress
Coordinate with team members for all study steps, evaluations, data collection and database requirements	1	Completed
Finalize assessment measurements	1	Completed
Milestone Achieved: 1st participant consented, screened and enrolled	1	Completed
Milestone Achieved: Study begins	1	Completed
Subject recruitment	1-12	In Progress
Participants evaluated pre and post surgical repair. N=60 randomly assigned to Group 1 (treatment) or Group 2 (control)	1-12	In Progress
Milestone Achieved: 60 patients enrolled	12	In Progress
Subtask 2: Follow-up and Interim Safety Analysis		Future
Complete follow-up assessments 12 months post-surgical intervention	13-24	Future
Perform safety analysis every 10 patients enrolled	As needed	Completed and continues to occur
Milestone Achieved: Statistical Analysis shows no adverse response to treatment.	As Available, every 10 pts	Completed and continues to occur
Major Task 4: Data Analysis		
Subtask 1: Coordinate with study personnel to finalize data collection and data quality		Future
Perform all analyses according to specifications, share output and finding with all investigators	25-30	Future
Dissemination of findings (abstracts, presentation, publications, DOD)	25-36	Future

Milestone Achieved: Report results from data analyses	25-36	Future
Subtask 2: Data Analysis		Future
Final data analysis	25-36	Future
Milestone Achieved: Report	25-36	Future

Specific Aim 2: Determine if dual coaptation site delivery of PEG with autografts is superior to autograft alone for segmental defects in human upper extremity injuries.	**Timeline**	**Research Sites**
	Months	**VUMC**
Major Task 1: Approvals from regulatory agencies.		
Subtask 1: Prepare Regulatory Documents and Research Protocol for Study		
Review IRB documents and confirm approval status	1	Completed
Submit any changes to VUMC Central IRB review	1	Completed
Submit for USAMRDC review (ORP/HRPO)	1	Completed
Review IND documents and confirm approval status	1	Completed
Clinicaltrial.gov registration	1	Completed
Submit amendments, adverse events and protocol deviations as needed	As Needed	Completed
Coordinate with IRB for annual continuing review	Annually	Completed
Milestone Achieved: Local IRB approval through VUMC	1	Completed
Milestone Achieved: HRPO approval for all protocols	1	Completed
Subtask1: Hiring and Training of Study Staff		
Advertise and interview for TBD project related staff	1-3	Completed
Coordinate hiring and training TBD staff	1-3	Completed
Milestone Achieved: Research staff trained	1-4	Completed
Major Task 3: Participant Recruitment, Intervention, Follow-up/Participant Evaluation		
Subtask 1: Recruitment		In progress
Coordinate with team members for all study steps, evaluations, data collection and database requirements	1	Completed
Finalize assessment measurements	1	Completed
Milestone Achieved: 1st participant consented, screened and enrolled	1	Completed
Milestone Achieved: Study begins	1	Completed
Subject recruitment	1-18	In progress
Participants evaluated pre and post surgical repair. N=60 randomly assigned to Group 1 (treatment) or Group 2 (control)	1-18	In progress
Milestone Achieved: 60 patients enrolled	18	In progress
Subtask 2: Follow-up and Interim Safety Analysis		Future
Complete follow-up assessments 12 months post-surgical intervention	25-42	Future
Perform safety analysis every 10 patients enrolled	As needed	Completed and continues to occur
Milestone Achieved: Statistical Analysis shows no adverse response to treatment.	As available, every 10 pts	Completed and continues to occur
Major Task 4: Data Analysis		

Subtask 1: Coordinate with study personnel to finalize data collection and data quality		Future
Perform all analyses according to specifications, share output and finding with all investigators	25-42	Future
Dissemination of findings (abstracts, presentation, publications, DOD)	25-48	Future
Milestone Achieved: Report results from data analyses	25-48	Future
Subtask 2: Data Analysis		Future
Final data analysis	25-48	Future
Milestone Achieved: Report	25-48	Future

Describe the Regulatory Protocol and Activity Status (if applicable).

Describe the Protocol and Activity Status for sections a-c, as applicable, using the format described for each section. If there is nothing significant to report during this reporting period, state "Nothing to Report."

(a) ***Human Use Regulatory Protocols***

TOTAL PROTOCOLS: 1

PROTOCOL (1 of 1 total):

Protocol [HRPO Assigned Number]: E02442.1a

Title: Nerve Repair Using Hydrophilic Polymers to Promote Immediate Fusion of Severed Axons and Swift Return of Function

Target required for clinical significance: 120 subjects

Target approved for clinical significance: 20 subjects

SUBMITTED TO AND APPROVED BY:

- **None**

STATUS:

(i) Number of subjects recruited/original planned target: 64/120
Number of subjects screened/original planned target: 64/120
Number of patients consented/original planned target: 18/120
Number of patients enrolled/original planned target: 14/120
Number of patients completed/original planned target: 0/20

NB: Of the *patients consented*, there were 6 patients who had to be withdrawn by the study team, including four patient who did not have sufficient nerve damage upon intraoperative investigation to satisfy inclusion criteria (eg nerve was not completely transected), one patient with too severe nerve injury to meet inclusion criteria (eg crush/injured segment distance too long), and one patient where the injury was deemed too distal by the PI (eg injury at fingertip, such that injury is equal to or less than 20 mm from the fingertip, precludes postoperative testing given that we perform two-point discrimination tests that are this size). Thus *patients enrolled* represents patients currently still within the clinical trial, ie 18-4=14 patients.

(ii) Report amendments submitted to the IRB and USAMRMC HRPO for review: None

(iii) Adverse event/unanticipated problems involving risks to subjects or others and actions or plans for mitigation: • None

(b) Use of Human Cadavers for Research Development Test & Evaluation (RDT&E), Education or Training

- No cadavers will be performed to complete the Statement of Work

(c) Animal Use Regulatory Protocols
TOTAL PROTOCOL(S):

- No animal use research will be performed to complete the Statement of Work

What do you plan to do during the next reporting period to accomplish the goals and objectives?

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

- Continue recruiting subjects
- Continue tracking enrolled subjects

Products: List any products resulting from the project during the reporting period. If there are no products to report for the current quarter, state "Nothing to report."

- Nothing to report

Participants & Other Collaborating Organizations

What individuals have worked on the project?
Provide the following information for: (1) Project Directors (PDs)/ PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort).

Name:	Wesley Thayer
Project Role:	Associate Professor
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	1.2
Contribution to Project:	Dr Thayer has assisted with ensuring study is launched according to protocol, as well as performing some of the operative procedures of the enrolled patients.
Funding Support:	Vanderbilt Department of Plastic Surgery and this grant

Name:	Alonda Pollins
Project Role:	Senior Research Specialist
Researcher Identifier (e.g. ORCID ID):	0000-0002-8331-7665
Nearest person month worked:	0.8
Contribution to Project:	Ms Pollins has assisted with ensuring study materials are adequately stocked and that participating team members are appropriately credentialled. During this interim, she transitioned to another department and her role will be replaced by Dr Ling Yan listed below.
Funding Support:	Vanderbilt Department of Plastic Surgery and this grant

Name:	Ling Yan
Project Role:	Senior Research Specialist
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	0.1
Contribution to Project:	Dr. Yan has been trained on the protocol and is in the process of completing electronic medical record training. She will assume the responsibilities of Alonda Pollins on this grant.
Funding Support:	

Name:	Christopher Kalmar
Project Role:	Clinical Research Fellow
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	2.3
Contribution to Project:	Dr Kalmar has assisted with subject enrollment, operating room protocol guidance, and postoperative clinical testing of nerve repair outcomes.
Funding Support:	Vanderbilt Department of Plastic Surgery and this grant

Name:	Jacqueline Oh
Project Role:	Clinical Research Coordinator
Researcher Identifier (e.g. ORCID ID):	*N/A*
Nearest person month worked:	10.26
Contribution to Project:	Ms Oh has assisted with subject enrollment, operating room protocol guidance, and postoperative clinical testing of nerve repair outcomes. She has departed the institution during this previous quarter and her role will be replaced by Sriya Nemani listed below.
Funding Support:	Vanderbilt Department of Plastic Surgery and this grant

Name:	Sriya Nemani
Project Role:	Clinical Research Coordinator
Researcher Identifier (e.g. ORCID ID):	*N/A*
Nearest person month worked:	1.8
Contribution to Project:	Ms Nemani has assisted with subject enrollment, operating room protocol guidance, and postoperative clinical testing of nerve repair outcomes.
Funding Support:	Vanderbilt Department of Plastic Surgery and this grant

Name:	Julia Yao
Project Role:	Research Nurse Practitioner
Researcher Identifier (e.g. ORCID ID):	*N/A*
Nearest person month worked:	6.7
Contribution to Project:	Ms Yao has assisted with subject enrollment and ensuring the study was launched according to protocol
Funding Support:	Vanderbilt Department of Plastic Surgery and this grant

Name:	Helen Ismail
Project Role:	Clinical Research Coordinator
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	8
Contribution to Project:	Ms Ismail has assisted with postoperative clinical testing of nerve repair outcomes
Funding Support:	Vanderbilt Department of Plastic Surgery and this grant

Name:	Isaac Manzanera Esteve
Project Role:	Co-Investigator
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	3.8
Contribution to Project:	Dr Manzanera has assisted with ensuring the study was launched according to protocol
Funding Support:	Vanderbilt Department of Plastic Surgery and this grant

Name:	Hakmook Kang
Project Role:	Co-Investigator

Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	0.6
Contribution to Project:	Dr. Kang provides statistical support for the project.
Funding Support:	

a. Actual Problems or delays and actions to resolve them

- Nothing to Reports

b. Anticipated Problems/Issues

- Nothing to Reports

Quad Charts: If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.

- Attached

KEY RESEARCH ACCOMPLISHMENTS

During the first year of the study, we have found that our institution was able to successfully deploy this study in the clinical setting, including approval of the study protocol by governing bodies, reliable referral of patients for enrollment, acquisition of required supplies and study drugs, implementation of the study protocol into the intraoperative workflow, reliable follow-up of patients for postoperative study assessments, and safety of the study drug.

REPORTABLE OUTCOMES

No adverse events. Other study endpoints are not yet ready to analyze.

CONCLUSIONS

The present study has been effectively and safely deployed at our institution. We look forward to further patient enrollment in the coming year, as well as beginning to analyze preliminary results after enough patients have been accrued with adequate follow-up duration.

REFERENCES

1. Wolfe SW, Hotchkiss RN, Pederson WC, Kozin SH. Green's Operative Hand Surgery. Churchill Livingstone. 2011; 5.

2. Kang JR, Zamorano DP, Gupta R. Limb salvage with major nerve injury: current management and future directions. J Am Acad Orthop Surg. 2011;19 Suppl 1:S28–34.

3. Holcomb JB, McMullin NR, Pearse L, Caruso J, Wade CE, Oetjen-Gerdes L, et al. Causes of death in U.S. Special Operations Forces in the global war on terrorism: 2001-2004. Annals of Surgery. 2007 Jun.;245(6):986–991.

4. Kragh JFJ, Walters TJ, Baer DG, Fox CJ, Wade CE, Salinas J, et al. Survival with emergency tourniquet use to stop bleeding in major limb trauma. Ann. Surg. 2009

Jan.;249(1):1–7.

5. Isaacson BM, Weeks SR, Pasquina PF, Webster JB, Beck JP, Bloebaum RD. The road to recovery and rehabilitation for injured service members with limb loss: a focus on Iraq and Afghanistan. US Army Med Dep J. 2010 Jul-Sep;:31–36.

6. Cross JD, Ficke JR, Hsu JR, Masini BD, Wenke JC. Battlefield orthopaedic injuries cause the majority of long-term disabilities. J Am Acad Orthop Surg. 2011;19 Suppl 1:S1–7.

7. Campbell WW. Evaluation and management of peripheral nerve injury. Clinical Neurophysiology. 2008 Sep.;119(9):1951–1965.

8. Stanec S, Tonković I, Stanec Z, Tonković D, Dzepina I. Treatment of upper limb nerve war injuries associated with vascular trauma. Injury. 1997 Sep.;28(7):463–468.

9. Lee SK, Wolfe SW. Peripheral nerve injury and repair. J Am Acad Orthop Surg. 2000 Jul-Aug;8(4):243–252.

10. Koeppen AH. Wallerian degeneration: history and clinical significance. J. Neurol. Sci. 2004 May 15;220(1-2):115–117.

11. Lunn E, Brown M, Perry V. The Pattern of Axonal Degeneration in the Peripheral Nervous-System Varies with Different Types of Lesion. Neuroscience. 1990;35(1):157–165.

12. Glass J, Culver D, Levey A, Nash N. Very early activation of m-calpain in peripheral nerve during Wallerian degeneration. J. Neurol. Sci. 2002;196:9–20.

13. Neumann B, Nguyen KCQ, Hall DH, Ben-Yakar A, Hilliard MA. Axonal regeneration proceeds through specific axonal fusion in transected C. elegans neurons. Dev. Dyn. 2011 Jun.;240(6):1365–1372.

14. Deriemer SA, Elliott EJ, Macagno ER, Muller KJ.Morphological evidence that regenerating axons can fuse with severed axon segments. Brain Res. 1983 Aug. 1;272(1):157–161.

15. Lore AB, Hubbell JA, Bobb DS, Ballinger ML, Loftin KL, Smith JW, et al. Rapid induction of functional and morphological continuity between severed ends of mammalian or earthworm myelinated axons. J. Neurosci. 1999 Apr. 1;19(7):2442–2454.

16. Britt JM, Kane JR, Spaeth CS, Zuzek A, Robinson GL, Gbanaglo MY, et al. Polyethylene glycol rapidly restores axonal integrity and improves the rate of motor behavior recovery after sciatic nerve crush injury. J. Neurophysiol. 2010 Aug.;104(2):695–703.

17. Spaeth C, Robinson T, Fan J, Bittner G. Cellular mechanisms of plasmalemmal sealing and axonal repair by polyethylene glycol and methylene blue. J. Neurosci. Res.

18. Bittner G, Keating C, Kane J, Britt J, Spaeth C, Fan J, et al. Rapid, effective and long-lasting behavioral recovery produced by microsutures, methylene blue and polyethylene glycol after complete cut of rat sciatic nerves. J. Neurosci. Res.

19. Kohler G, Milstein C. Continuous cultures of fused cells secreting antibody of predefined specificity. Nature. 1975 Aug. 7;256(5517):495–497.

20. Ramon Y, Cajal S. Degeneration & Regeneration of the Nervous System. Translated and edited by Raoul M. May. Hafner Publishing Co.; 1928.

21. Nguyen MP, Bittner GD, Fishman HM. Critical interval of somal calcium transient after neurite transection determines B 104 cell survival. J. Neurosci. Res. 2005 Sep. 15;81(6):805–816.

22. Krause TL, Bittner GD. Rapid morphological fusion of severed myelinated axons by polyethylene glycol. Proc Natl Acad Sci U S A. 1990 Feb.;87(4):1471–1475.

APPENDIX 1 Financial

Thayer: OR200053
W81XWH-21-1-0441/Nerve Repair with Polyethylene Glycol to Promote Rapid Return of Nerve Function

Year 1	07/01/21-06/30/22	To date through 06/30/2022		
		Budget	Actual	Balance
	Personnel	$		$
	Supplies	$	$	$
	Travel	$	$	$
	Subcontract		$ -	$ -
	Indirects	$		$
	Total	$		$

Year 2	07/01/22-06/30/23			
		Budget	Actual	Balance
	Personnel	$	$ -	$
	Supplies	$	$	
	Travel	$		$
	Subcontract			$ -
	Indirects	$	$ -	$
	Total	$	$ -	$

Year 3	07/01/23-06/30/24			
		Budget	Actual	Balance
	Personnel	$		$
	Supplies	$		$
	Travel	$		$
	Subcontract			$ -
	Indirects	$		$
	Total	$ $	-	$

Year 4	07/01/24-06/30/25			
		Budget	Actual	Balance
	Personnel	$		$
	Supplies	$		$
	Travel	$		$
	Subcontract			$ -
	Indirects	$		$
	Total	$ $	-	$

Grant Project to Date Actual	Through September 2021		
	Budget	Actual	Balance
Personnel	$	$	$
Supplies	$	$	$
Travel	$	$	$
Subcontract	$ -		$ -
Indirects	$		$
Total	$		$

Budget Award Year	
1	$
Year 2	$
Year 3	$
Year 4	$