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TITLE: A Tacrolimus-Releasing Nanofiber Nerve Wrap to Enhance Motor and Sensory Recovery in Injured Peripheral Nerves

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13. SUPPLEMENTARY NOTES

14. ABSTRACT
Nerve injuries from penetrating or blunt trauma often result in life-long disability with loss of sensation and paralysis. Recovery is usually poor. A key barrier to recovery is the naturally slow axonal regeneration rate which consequently deprives the target muscle and skin of nerve supply and results in permanent loss of function. We aim to validate a reliable, effective, and easy-to-use, off-the-shelf product for local drug delivery following nerve surgery and demonstrate its effectiveness. We have therefore designed a biodegradable wrap for the delivery of tacrolimus that surgeons can place around the site of injured nerves during surgery. We will fabricate nerve wraps made from polymers sheets loaded with tacrolimus. In summary, we have begun development of the electrospun nerve wrap, loaded it with tacrolimus, and have started the validation for use in animal studies. This is significant since we are now ready to move forward with in vivo use in a peripheral nerve injury model.

15. SUBJECT TERMS
None listed.

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1. INTRODUCTION

Nerve injuries from penetrating or blunt trauma often result in life-long disability with loss of sensation and paralysis. Recovery is usually poor. A key barrier to recovery is the naturally slow axonal regeneration rate which consequently deprives the target muscle and skin of nerve supply and results in permanent loss of function. Drug-based therapies that accelerate axonal regeneration may decrease the denervation time and thereby improve functional recovery. However, no such treatments to augment the results of surgery are currently available. Local delivery of the transplant antirejection drug also known as tacrolimus via biodegradable gels to the nerve repair site results in enhanced nerve regeneration. A more convenient, surgeon-friendly delivery system is needed for clinical use in the operating room. We aim to validate a reliable, effective, and easy-to-use, off-the-shelf product for local drug delivery following nerve surgery and demonstrate its effectiveness. We have therefore designed a biodegradable wrap for the delivery of tacrolimus that surgeons can place around the site of injured nerves during surgery. We will fabricate nerve wraps made from polymers sheets loaded with tacrolimus. Based on our preliminary studies on gel-based application of tacrolimus, these devices will provide sustained tacrolimus release to the nerve repair site for four weeks or longer. We will assess effectiveness in a rat model by wrapping the devices around nerve repair sites. Adult rats will undergo hindlimb nerve transection and immediate repair. Intraoperatively, rats will receive either the nerve wrap loaded with tacrolimus (local treatment), an empty nerve wrap without tacrolimus, daily subcutaneous tacrolimus injections, or undergo nerve repair only as a control group. After 21 days, we will assess the number of sensory and nerve cells that regenerate to the skin and muscle using tracer dyes. To determine the effect of local tacrolimus delivery on recovery of movement rats will undergo unilateral median (forearm) nerve cut and repair and will be randomly allocated to tacrolimus nerve wrap treatment or vehicle alone. We anticipate that the devices will increase the number of nerve regeneration to the muscle and skin, resulting in improved and accelerated functional recovery. The results will form the basis for human device trials to enhance outcomes following nerve trauma.

2. KEYWORDS: peripheral neuropathy, nerve wrap, sustained release drug delivery, nerve repair

3. ACCOMPLISHMENTS

What were the major goals of the project?

The major goals of this project are to assess the efficacy of an electrospun, tacrolimus-releasing nerve wrap on three nerve injury paradigms:

1) Acute cut and repair of the common peroneal nerve
2) Transection and delayed repair
3) Transection and repair using a nerve graft model

What was accomplished under these goals?

Due to the nature of electrospinning an implantable medical device, reproducibility and safety are of great importance. Prior to beginning work on this project, this lab underwent a relocation to a new facility which required re-implementation of the devices used for electrospinning, as well as identification of new systems to support safe application in the planned animal model.

1) Major activities
    a. Electrospinning has been successful implemented in a novel lab setting (NanoSpinner, Inovenso Technology Inc, Boston, MA, USA), and physical characterization of the tacrolimus nerve wrap to date has shown comparable results to prior nanofiber mesh fabrication.
        i. Polycarbonate urethane (PCNU) has been successfully synthesized through collaboration with a biomedical engineering team at the University of Toronto.

ii. Due to the cost and difficulty of sourcing PCNU, preliminary trials with a test polymer, poly lactic-co-glycolic acid (PLGA) were performed to test feasibility and suitable operation of the newly implemented system.
iii. Characterization of nanofiber morphology was performed by mounting and sputter coating with gold prior to imaging using a JSM-7800F Schottky Field Emission Scanning Electron Microscope (JEOL, Japan).
iv. Determination of average fiber diameter and porosity of SEM-produced images was carried out via the ImageJ plugin, DiameterJ (NIH, https://imagej.nih.gov/ij/)
v. Uniaxial trials using PCNU polymer showed similar reproducibility to PLGA, and characterization of these fibers via scanning electron microscopy showed results comparable to prior work.
vi. Coaxial trials using PCNU and 22 and 18-gauge blunt-tip needles were carried out in batches, with suitable reproducibility to prior electrospun wraps upon SEM evaluation of nanofiber morphology (mean fiber diameter 0.438 μm, 60.19% porosity)
vii. Coaxial trials of tacrolimus-infused inner core fibers were carried out, and drug inclusion was determined to not affect solubility or reproducibility of nanofibers in the fabrication of a nanofiber mesh for use as a nerve wrap.

b. Protocol optimization for histomorphometric analysis is underway, and test samples are awaiting imaging by TEM through the electron microscopy core.
c. Protocol optimization has been performed for measurement of muscle force in a median nerve injury and repair model through forepaw grip function tests.
d. In-vitro release media from synthesized nerve wraps is currently being collected for analysis of encapsulation efficiency, as well as for use in a dorsal root ganglia neurite extension assay to prove a suitable release profile of newly fabricated wraps prior to in-vivo experimentation.
e. Sterilization of collected electrospun fiber mats is planned to allow for implantation into transected rat nerve repairs according to the treatments outlined in the objectives above

2) Specific Objectives
   a. A tacrolimus-releasing nerve wrap has been successfully fabricated with characterization results thus far comparable to those of prior work.
   b. Protocols have been optimized to move forward with animal model implementation upon resolution of sterilization challenges in wraps to be placed.

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

This project has provided the basis for collaboration between lab members and faculty and students at

- The Institute of Biomedical Engineering at the University of Toronto
- The Integrated Nanosystems Development Institute at Indiana University-Purdue University Indianapolis
- The Department of Intelligent Systems Engineering at Indiana University Bloomington.

Discussions with and one-on-one training from individuals in these groups has benefitted those members of our research team involved with this project by introducing key concepts and spurring discussions of other advancements within the field.

How were the results disseminated to communities of interest?

Concepts of this project have been published in one peer-reviewed articles, presented as two posters at a departmental research event, and will be presented at a local surgical conference.

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

Upon resolution of wrap sterilization challenges, animal model implementation will begin. Specifically, animals selected to undergo delayed cut and repair will receive common peroneal nerve transection to allow sufficient time to pass prior to repair and wrap placement. During this time, rats selected for use in Objective 1 will receive transection and immediate repair with one of four conditions: tacrolimus-loaded nerve wrap, vehicle nerve wrap, tacrolimus subcutaneous injection, or vehicle injection.

Rats will be sacrificed at various postoperative timepoints, and nerve sections will be analyzed by previously optimized histomorphometry protocols.

Daily postoperative forepaw grip function will be assessed to determine time to return of active finger flexion in each treatment group.

Plasma and organ tacrolimus drug levels will be assessed at various timepoints throughout objectives 1-3.

4. IMPACT

What was the impact on the development of the principal discipline(s) of the project?

The development of a drug-releasing nerve wrap that is biodegradable, biocompatible, and easy to adjust in terms of drug loaded and its dosage presents a myriad of opportunities in the administration of therapeutics that at present are limited by systemic toxicity. Its component compounds at the time of application allow for placement at any site requiring a small to medium-sized planar or cylindrical surface that can be anchored too intraoperatively.

What was the impact on other disciplines?

Nothing to report.

What was the impact on technology transfer?

Nothing to report.

What was the impact on society beyond science and technology?

Aspects of this project have been frequently discussed by the lab member leading the project at an underserved high school in the area during programming designed to improve scientific literacy and compel students to pursue higher education and training in medical sciences.

5. CHANGES/PROBLEMS

Changes in approach and reasons for change.

During initial implementation of our electrospinning apparatus, the use of a test polymer, PLGA, was recommended before moving to PCNU. PLGA is more readily available and cost-effective, though less optimal for in-vivo implantation. This was performed, and electrospinning has since been successful using PCNU as the experimental drug-infused polymer.

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

A delay was experienced in the initial phases of this project due to changes in the individual leading it, as well as a relocation to a new facility. This required manufacturer, electrician, and facility staff intervention to ensure the electrospinner could be used optimally and safely.

Changes that had a significant impact on expenditures.

Nothing to report.

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

Nothing to report.

Board/Institutional Animal Care and Use Committee approval dates.

Nothing to report.

Significant changes in use or care of human subjects.

Nothing to report.

Significant changes in use or care of vertebrate animals.

Nothing to report.

Significant changes in use of biohazards and/or select agents.

Nothing to report.

6. PRODUCTS

Publications, conference papers, and presentations

Publications

- Daeschler, Simeon C; So, Katelyn J.W.; Feinberg, Konstantin; Manoraj, Marina; Cheung, Jenny; Zhang, Jennifer; Mirmoeini, Kaveh; Santerre, J. Paul; Gordon, Tessa; Borschel, Gregory H. A functional tacrolimus-releasing nerve wrap for enhancing nerve regeneration following surgical nerve repair. Neural Regeneration Research (): 10.4103/NRR.NRR-D-22-01198, January 31, 2024. | DOI: 10.4103/NRR.NRR-D-22-01198

Presentations

- "Essential considerations in the design of a drug-releasing nerve wrap for mass manufacturing and widespread clinical implementation," Plastic Surgery Research Day, Indiana University Department of Surgery, Indianapolis, IN, January 2024
- "The Gap Between Plans and Reality: Addressing Challenges in Electrospinning," Plastic Surgery Research Day, Indiana University Department of Surgery, Indianapolis, IN, January 2024
- "A drug-releasing nerve wrap to enhance nerve regeneration," 71st Annual Scientific Meeting, Indiana Chapter of the American College of Surgeons, Noblesville, IN, April 2024

Website(s) or other Internet site(s)

Nothing to report.

Technologies or techniques

We have successfully electrospun the nanofiber nerve wrap and have loaded the wrap with tacrolimus. We are currently validating the nerve wrap with in vitro studies and scanning electron microscopy.

Inventions, patent applications, and/or licenses

Nothing to report.

Other Products

Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on this project?

Name:	Gregory Borschel, MD
Project Role:	Principal Investigator
Researcher Identifier (ORCID ID):	0000 0001 7691 5264
Nearest person month worked:	14
Contribution to Project:	Dr. Borschel is the principal investigator of this project. He provides oversight and guides project experimental design, analysis, and troubleshooting.

Name:	Konstantin Feinberg, PhD
Project Role:	Co-Investigator
Researcher Identifier (ORCID ID):	0000 0001 7565 4680
Nearest person month worked:	14
Contribution to Project:	Dr. Feinberg helped performed the validation of the nerve wrap in vitro. Additionally, he provided guidance over the project experimental design and analysis.

Name:	Paul Santerre, PhD
Project Role:	Collaborator
Researcher Identifier (ORCID ID):	0000 0003 3373 6463
Nearest person month worked:	3
Contribution to Project:	Dr. Santerre provided training in his lab for our graduate student and research fellow to use the electrospinner. Additionally, his lab provided PCNU for use in our lab in Indiana.

Name:	Mario Henriquez
Project Role:	Laboratory Manager
Researcher Identifier (ORCID ID):	0000 0003 2881 6151
Nearest person month worked:	12
Contribution to Project:	Mr. Henriquez provided the logistical support for the project, including ordering supplies, writing reports, and coordinating collaborations and travel to other sites as needed.

Name:	Jordan Crabtree
Project Role:	Graduate Student
Researcher Identifer (ORCID ID):	0000 0002 1157 4873
Nearest person month worked:	10
Contribution to Project:	Mr. Crabtree extensively worked on re-validating the nerve wrap at Indiana University. He has electrospun the wraps, loaded the wraps with drugs, and is currently validating the nerve wraps at an electron microscopy core.
Funding Support:	MedSTAR Program at Indiana University School of Medicine

Name:	Chilando Mulenga
Project Role:	Research Fellow
Researcher Identifer (ORCID ID):	N/A
Nearest person month worked:	5
Contribution to Project:	Mr. Mulenga aided Mr. Crabtree on the re-validation of the nerve wrap.
Funding Support:	Startup funds from Indiana University

Name:	Arif Hussain, MD
Project Role:	Postdoctoral researcher
Researcher Identifer (ORCID ID):	N/A
Nearest person month worked:	2
Contribution to Project:	Dr. Hussain provided some assistance to Mr. Crabtree on the re-validation of the nerve wrap.
Funding Support:	Startup funds from Indiana University

What other organizations were involved as partners?

Organization Name:	Dr. Paul Santerre's Lab at the University of Toronto
Location of Organization:	661 University Ave, M5G, 1M1, Toronto, CA
Partner's contribution to the project	
Facilities:	Provided a training space for use of the electrospinner at the Institute of Biomedical Engineering
Collaboration:	Alongside Dr. Santerre's students, Mr. Brian Webb and Ms. Katya D'Costa, they provided guidance and hands on training on use of the electrospinner and creating PCNU nerve wraps.

Organization Name:	The Integrated Nanosystems Development Institute at Indiana University-Purdue University Indianapolis
Location of Organization:	723 W. Michigan St, Indianapolis, IN, 46202-5195
Partner's contribution to the project	
Facilities:	Provided training space and use of the scanning electron microscope.
Collaboration:	Provided a protocol for use of the scanning electron microscope in their core.

Organization Name:	The Department of Intelligent Systems Engineering at Indiana University Bloomington.
Location of Organization:	700 N Woodlawn Ave, Bloomington, IN 47408
Partner's contribution to the project	
Collaboration:	Provided guidance and assistance for the validation of the nerve wrap with the scanning electron microscope. Additionally, provided guidance and assistance in the electrospinning.

8. SPECIAL REPORTING REQUIREMENTS

Collaborative Awards

Nothing to report.

Quad Chart

Attached as a separate document.

9. APPENDICES

A link to the manuscript "A functional tacrolimus-releasing nerve wrap for enhancing nerve regeneration following surgical nerve repair" can be found below:

https://journals.lww.com/nrronline/abstract/9900/a_functional_tacrolimus_releasing_nerve_wrap_for.204.aspx

9. APPENDICES CONT.

Abstracts:

1. Presented at Plastic Surgery Research Day, Indiana University Department of Surgery, Indianapolis, IN, January 2024

Essential considerations in the design of a drug-releasing nerve wrap for mass manufacturing and widespread clinical implementation

Jordan R Crabtree, B.S., Chilando Mulenga, B.S., Mario Henriquez, B.S., Arif Hussain, MD, Khoa Tran, MPH, Konstantin Feinberg, PhD, Gregory Borschel, MD

Introduction

The application of core-shell nanofibers infused with locally acting therapeutics represents a promising avenue for avoiding the systemic toxicity of many compounds. Applying this concept in the development of a drug-releasing nerve wrap is a novel approach to optimizing the fields of nerve repair and regeneration. Tacrolimus is an FDA-approved calcineurin inhibitor, and polyurethane plastics such as polycarbonate-urethane (PCNU) are currently employed in many FDA-approved implantable medical devices. While no objective outcome can serve as a surrogate for manufacturability or the likelihood of adoption by surgeons, a variety of properties deserve consideration in the design of our proposed biodegradable and biocompatible implantable medical device. This study sought to identify these properties and commonly employed methods to address them.

Methods

To ensure our design of a tacrolimus-releasing, PCNU nerve wrap is optimized for both manufacturability and clinical use, we identified the following characteristics in choosing our polymer and drug combination: regulatory agency compliance, shelf-life adequacy, withstanding of sterilization practices, and ease of surgical implementation.

Results

Thermal Gravimetric Analysis and Differential Scanning Calorimetry have shown that tacrolimus and electrospun PCNU are stable at both ambient and physiological temperatures. Sterilization can be carried out without altering the structural integrity of the wrap or drug through the process of gamma-irradiation.

Conclusion

In the design of a novel implantable medical device, there are many factors to consider outside of efficacy in in-vitro and/or in-vivo experiments. Our work combines existing translational science concepts with large-scale manufacturing principles to address the challenges of approval and subsequent broad implementation of our final product. Finally, a major benefit of the use of an off-the-shelf drug-releasing nerve wrap is ease of use, as well as surgeon familiarity with existing nerve wraps currently employed in nerve repair.

9. APPENDICES CONT.

2. Presented at Plastic Surgery Research Day, Indiana University Department of Surgery, Indianapolis, IN, January 2024

The Gap Between Plans and Reality: Addressing Challenges in Electrospinning

Jordan R Crabtree, B.S., Chilando Mulenga, B.S., Mario Henriquez, B.S., Arif Hussain, MD, Khoa Tran, MPH, Konstantin Feinberg, PhD, Gregory Borschel, MD

Introduction:

Electrospinning is a nanofiber fabrication technique often employed in the field of biomedical engineering. Its practical application entails unforeseen challenges that are often left undiscussed and, therefore, unconsidered by those outside of this specialized field. This study aims to elucidate the challenges faced in implementing electrospinning in a lab setting, with particular emphasis on challenges that arose that were difficult to predict by literature review alone.

Methods:

A literature review was conducted to compile known challenges in assembling and implementing an electrospinning apparatus. Electrospinning of a test polymer, Poly Lactic-co-glycolic Acid (PLGA), was conducted. Challenges specific to this implementation were carefully recorded with regard to equipment compatibility, safety, and other considerations not found in the literature. These challenges were subsequently addressed in consultation with facility personnel and external research groups.

Results:

Prior to running any trials, our first issue was ensuring a safe environment for the preparation and housing of our equipment, which required intervention by facility electricians. We next experienced difficulties in sourcing a test polymer and solvent with proven results at defined concentrations and viscosities.

After safe operation was established and a test polymer was obtained, uniaxial trials were successful. However, coaxial trials revealed incompatibilities between our electrospinning setup and our chosen polymers. This included our solvent reacting with our tubing and sealing devices, which required further modification to our equipment.

Conclusions:

This study provides a detailed report of the underreported challenges faced by our team in electrospinning, particularly in safety and equipment compatibility. It underscores the importance of careful planning and maintaining a low threshold of consulting experts in implementing novel techniques in untested environments. By bringing light to these issues, we hope to lessen the divide between two disciplines working in parallel to optimize this technique for application in translational research and better patient outcomes.

9. Will be presented at 71st Annual Scientific Meeting, Indiana Chapter of the American College of Surgeons, Noblesville, IN, April 2024

A drug-releasing nerve wrap to enhance nerve regeneration

Jordan R Crabtree, B.S., Chilando Mulenga, B.S., Mario Henriquez, B.S., Arif Hussain, MD, Khoa Tran, MPH, Konstantin Feinberg, PhD, Gregory Borschel, MD

Learning Objective

A tacrolimus-releasing nerve wrap improves nerve healing, and many factors will determine its potential for clinical integration.

Introduction

The application of core-shell nanofibers infused with locally acting therapeutics represents a promising avenue for avoiding the systemic toxicity of many compounds. Applying this concept in the development of a drug-releasing nerve wrap is a novel approach to optimizing the fields of nerve repair and regeneration. Tacrolimus is an FDA-approved calcineurin inhibitor with evidence suggesting it improves nerve healing, and polyurethane plastics such as polycarbonate-urethane (PCNU) are currently employed in many FDA-approved implantable devices. While no objective outcome can serve as a surrogate for manufacturability or the likelihood of adoption by surgeons, a variety of properties deserve consideration in the design of our proposed biodegradable and biocompatible wrap. This study sought to identify these properties and commonly employed methods to address them.

Methods

To ensure our design of a tacrolimus-releasing, PCNU nerve wrap is optimized for both manufacturability and clinical use, we identified the following characteristics in choosing our polymer and drug combination: regulatory agency compliance, shelf-life adequacy, withstanding of sterilization practices, and ease of surgical implementation.

Results

A Bioactivity Assay using rat dorsal root ganglia shows that Tacrolimus increases neurite extension in vitro. Thermal Gravimetric Analysis and Differential Scanning Calorimetry have shown that tacrolimus and electrospun PCNU are stable at both ambient and physiological temperatures. Sterilization can be carried out without altering the structural integrity of PCNU or Tacrolimus through the process of gamma-irradiation.

Conclusion

In the design of a novel implantable medical device, there are many factors to consider outside of efficacy in in-vitro and/or in-vivo experiments. Our work combines existing translational science concepts with large-scale manufacturing principles to address the challenges of approval and subsequent broad implementation of our final product. Finally, a major benefit of the use of an off-the-shelf drug-releasing nerve wrap is ease of use, as well as surgeon familiarity with existing nerve wraps currently employed in nerve repair.