

AWARD NUMBER: W81XWH-16-1-0662

TITLE: Side-to-Side Supercharging Allograft

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CONTRACTING ORGANIZATION: Virginia Commonwealth University
Richmond, VA 23284

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

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14. ABSTRACT Side-to-Side bridge grafting into processed acellular nerve graft will improve the neurotrophic environment and stimulate Schwann cells to improve axon regeneration and increase the effective "critical length" of allograft (PNA).						
15. SUBJECT TERMS Supercharging of Nerves, Isograft, Processed Acellular Nerve Allograft, Bridge Graft						
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- 1. INTRODUCTION:** Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.

Processed acellular nerve allograft (PNA) is becoming an increasingly popular modality for addressing segmental nerve defects during nerve repair. Side-to-side grafting between the PNA and regional in situ nerve trunks may be able to increase the effective “critical length” of the PNA. Nerve tissue loss and retraction can result in segmental gaps requiring some form of grafting. Autologous nerve grafting is associated with potential donor morbidity, added surgical time, and increased surgical effort. In some circumstances, such as may be seen in military level traumas, there is insufficient nerve autograft material available due to the complex multi extremity involvement. Processed Acellular Nerve Allograft (PNA) maintains some guidance cues and provides an effective scaffolding system but depends on in situ Schwann cell migration to support axon regeneration. Though this process appears reliable over short and medium nerve defects, with increasing graft lengths, Schwann cells regress from a neurosupportive phenotype to an inactive or senescent state in which proliferation, apoptosis, and normal function are all impaired. A similar process occurs in chronically denervated nerve in which Schwann cell numbers are decreased and regeneration-associated genes are downregulated. Recently, several small animal studies demonstrated that donor axons introduced into the side of a long autograft or chronically denervated nerve stump (by reverse end-to-side nerve repair or “supercharging”) enhanced axon regeneration and recovery. In a variation of this strategy, donor axons provided by “side-to-side” bridge grafts placed between an intact rodent tibial nerve and the chronically denervated distal peroneal stump (like rungs on a ladder) improved muscle weight, axon numbers, and myelination (implicating the Schwann cells) following delayed repair. Our hypothesis is: Side-to-side bridge grafting into processed acellular nerve allograft will improve the neurotrophic environment and stimulate Schwann cells to improve axon regeneration and increase the effective “critical length” of allograft (PNA).

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

Supercharging of nerves, Isograft, Processed Acellular nerve allograft, Bridge Graft

3. **ACCOMPLISHMENTS:** The 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.

What were the major goals of the project?

List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

Specific Aim 1: To determine if side-to-side nerve transfers will increase the number and length of axons regenerating across short and long nerve defects repaired with acellular nerve allograft.		
Major Task 1: Required regulatory review and approval process		
Subtask	Description	Status
1	Obtain IACUC and USAMRMC review and approval of all necessary animal use	100%
2	Order and obtain supplies necessary for experimentation	100%
3	Breeding and maintenance of Thy1-GFP Sprague-Dawley rats	100%
	Milestone Achieved: IACUC/ACURO Approval Breeding of Thy1-GFP Sprague-Dawley Rats	
Major Task 2: Perform rodent experiments utilizing PNA to create 0, 1, or 3 side-to-side bridge grafts to varying lengths (short and long) of both Isograft (Iso) and Processed acellular Nerve Allograft (PNA)		
Subtask	Description	Status
1	Animal Survival Surgeries for Batch 1 (18 Sprague-Dawley and 4 Thy1-GFP rats will undergo immediate repair of the transected nerve with 20mm (n=9 Sprague Dawley; n=2 Thy1-GFP) or 40mm (n=9 Sprague Dawley; n=2 Thy1-GFP) Isograft (Groups Iso20 and Iso40))	18.2% (4 out of 22 surgeries completed as of 10/25/17) 100% completed as of 02/26/2018
2	Animal Survival Surgeries for Batch 2 (18 Sprague-Dawley and 4 Thy1-GFP rats will undergo immediate repair of the transected nerve with 20mm (n=9 Sprague Dawley; n=2 Thy1-GFP) or 40mm (n=9 Sprague Dawley; n=2 Thy1-GFP) PNA (Groups PNA20 and PNA40))	27.3% (6 out of 22 surgeries completed as of 10/25/17) 100% completed as of 02/26/2018
3	Animal Survival Surgeries for Batch 3 (48 Sprague Dawley and 8 Thy1-GFP rats will undergo immediate repair of the transected nerve with 20mm (n=24 Sprague Dawley; n=4 Thy1-GFP) or 40mm (n=24 Sprague Dawley; n=4 Thy1-GFP) PNA combined with one (n=12 Sprague Dawley; n=2 Thy1-GFP) or three (n=12 Sprague Dawley; n=2 Thy1-GFP) side-to-side bridging nerve transfer(s)	28.6% (16 out of 56 surgeries completed as of

	from the peroneal nerve (Groups PNA20+1S, PNA20+3S, PNA40+1S, and PNA40+3S). The bridging side-to-side nerve grafts will be performed using 1 or 3 six mm long rodent PNA sutured to 1 or 3 perineurial windows (500 microns in diameter) in the side of the peroneal nerve and in the side of the graft material. The grafts will be positioned in a “C” or serpentine configuration into a subcutaneous pocket in the ipsilateral thigh so that the side-to-side bridge graft insertion points will be brought near the intact donor nerve (see Fig 2). All nerve coaptations will be performed with three to four 10-0 nylon stitches under surgical microscope magnification.)	10/25/17) 100% completed as of 02/26/2018
	Milestone(s) Achieved: Completion of all Survival Surgeries	
Major Task 3: Assess axon regeneration utilizing Thy1-GFP rats to visualize axon elongation from tibial nerve stump, across PNA or Isograft, into distal stump		
Subtask	Description	Status
1	Animal Terminal Surgeries for Batch 1 (4 Thy1-GFP rats (n=2 from Iso20 and Iso40 each) will undergo in-vivo imaging of nerves using fluorescence-enabled microscope utilizing GFP (488nm) fluorescent and bright field filters)	75% (completed as of 10/25/17)
2	Animal Terminal Surgeries for Batch 2 (4 Thy1-GFP rats (n=2 from PNA20 and PNA40 each) will undergo in-vivo imaging of nerves using fluorescence-enabled microscope utilizing GFP (488nm) fluorescent and bright field filters)	75% (completed as of 10/25/17)
3	Animal Terminal Surgeries for Batch 3 (8 Thy1-GFP rats (n=2 from PNA20+1S, PNA20+3S, PNA40+1S, and PNA40+3S each) will undergo in-vivo imaging of nerves using fluorescence-enabled microscope utilizing GFP (488nm) fluorescent and bright field filters)	75% (completed as of 10/25/17)
	Milestone(s) Achieved: Assessment of axon regeneration utilizing Thy1-GFP rats	
Major Task 4: Assess axon regeneration utilizing Sprague-Dawley rats and using muscle contraction force		
Subtask	Description	Status
1	Animal Terminal Surgeries for Batch 1 (18 Sprague Dawley rats (n=9 from Iso20 and Iso40 each) will undergo motor testing and tissue harvest for morphologic and histologic)	0%
2	Animal Terminal Surgeries for Batch 2 (18 Sprague Dawley rats (n=9 from PNA20 and PNA40 each) will undergo motor testing and tissue harvest for morphologic and histologic examination)	11.1% (2 out of 18 samples assessed as of 10/25/17)
3	Animal Terminal Surgeries for Batch 3 (36 Sprague Dawley rats (n=9 from PNA20+1S, PNA20+3S, PNA40+1S, and PNA40+3S each) will undergo motor testing and tissue harvest for morphologic and histologic examination)	22.2% (8 out of 36 samples assessed as of 10/25/17)
	Milestone(s) Achieved: Assessment of axon regeneration using muscle contraction force in Sprague Dawley rats	
Major Task 5: Assess axon regeneration utilizing Sprague-Dawley rats using nerve histology as		

end points		
Subtask	Description	Status
1	Nerve Samples from Batch 1 (prepare, section, stain, image, and analyze)	0%
2	Nerve Samples from Batch 2 (prepare, section, stain, image, and analyze)	2.2%
3	Nerve Samples from Batch 3 (prepare, section, stain, image, and analyze)	4.4%
	Milestone(s) Achieved: Assessment of axon regeneration using nerve histology of samples from Sprague Dawley rats	

Specific Aim 2: To determine if supercharging side-to-side nerve transfer decreases Schwann cell senescence in acellular nerve allograft.

Major Task 1: Evaluate for Schwann cell senescence using S100 immunostaining (stains Schwann cells) and β -gal immunostaining (senescence marker) at regular intervals across the length of the isografts and PNAs

Subtask	Description	Status
1	Nerve Samples from Batch 1 (prepare, section, stain, image, and analyze)	0%
2	Nerve Samples from Batch 2 (prepare, section, stain, image, and analyze)	2.2%
3	Nerve Samples from Batch 3 (prepare, section, stain, image, and analyze)	4.4%
	Milestone Achieved: Evaluation of Schwann Cell Senescence	

Specific Aim 3: To determine the effect of supercharging side-to-side transfer on Schwann cell function in acellular nerve allograft.

Major Task 1: Measure axon diameters and myelination in Isografts and PNAs treated with side-to-side nerve transfer

Subtask	Description	Status
1	Nerve Samples from Batch 1 (prepare, section, stain, image, and analyze)	0%
2	Nerve Samples from Batch 2 (prepare, section, stain, image, and analyze)	0%
3	Nerve Samples from Batch 3 (prepare, section, stain, image, and analyze)	0%
	Milestone Achieved: Measurements of axon diameter and myelination	

Specific Aim 4: To confirm improved regeneration related to Schwann cell stimulation and not to “donated” extra axons.

Major Task 1: Use retrograde labeling to quantify axons donated from peroneal nerve into recipient Isograft and PNA.

Subtask	Description	Status
1	12 Sprague Dawley rats (n=3 from PNA20+1S, PNA20+3S, PNA40+1S, and PNA40+3S each) from batch 3 will undergo back-labeling with retrograde fluorescent dyes	0%

2	Spinal Cord Samples (collect, prepare, section, image, and analyze)	0%
	Milestone Achieved: Understanding source of axonal regeneration	
Major Task 2: Evaluate overall outcome of the experiment by looking at all collected data together		
Subtask	Description	Status
1	Data Analysis and Discussion	Pending
2	Manuscript preparations	0%
	Milestone Achieved: Completion of the manuscript and final report	

What was accomplished under these goals?

For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

Specific Aim 1: Major Task 1: All regulatory requirements satisfied to allow for research work to start. Thy1-GFP animals bred to satisfy the needs for this study.

Specific Aim 1: Major Task 2: 26 out of 100 animal surgeries completed (26%) using Processed Nerve Allograft (PNA) were used to create 0, 1, or 3 side-to-side bridge grafts to varying lengths of both Isograft (Iso) and PNA.

As of 02/26/2018, 100 out of 100 animal surgeries completed (100%) using Processed Nerve Allograft (PNA) were used to create 0, 1, or 3 side-to-side bridge grafts to varying lengths of both Isograft (Iso) and PNA.

Specific Aim 1: Major Task 3: All 18 Thy1-GFP animals underwent in-vivo imaging of nerves using fluorescence-enabled microscope utilizing GFP (488nm) fluorescent and bright field filters. Images are currently undergoing analysis to quantify the amount of regeneration occurred in the graft and the source of the regeneration.

Specific Aim 1: Major Task 4: Muscle contraction force assessed in 10 out of 54 animals (18.5%). See appendix 2 for the data.

As of 02/26/2018, see appendix 3 for the data and appendix 4 for Results discussion.

Specific Aim 1: Major Task 5: 10 out of 72 nerve samples prepared to assess axonal regeneration using toluidine blue staining and axon analysis.

Specific Aim 2: Major Task 1: 10 out of 72 nerve samples prepared to assess state of Schwann Cells using s100 immunostaining (stains Schwann Cells) and β -gal immunostaining (senescence marker).

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.”

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. “Training” activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. “Professional development” activities result in increased knowledge or skill in one’s area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

Nothing to report.

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.

Nothing to report.

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

If this is the final report, state “Nothing to Report.”

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

We plan to complete following before the next reporting period (December 30, 2017):

Specific Aim 1: Major Task 2: 50 out of 100 animal surgeries completed (50%) using Processed Nerve Allograft (PNA) were used to create 0, 1, or 3 side-to-side bridge grafts to varying lengths of both Isograft (Iso) and PNA.

Specific Aim 1: Major Task 3: Complete analyzing images in effort to quantify the amount of regeneration occurred in the graft and the source of the regeneration.

Specific Aim 1: Major Task 5: Sectioning of 10 nerve samples prepared to assess axonal regeneration using toluidene blue staining and axon analysis.

Specific Aim 2: Major Task 1: Sectioning of 10 nerve samples prepared to assess state of Schwann Cells using s100 immunostaining (stains Schwann Cells) and β -gal immunostaining (senescence marker).

We plan to complete most of the tasks mentioned in the Statement of Work by next Annual reporting period and we will send the No Cost Extension request soon.

4. **IMPACT:** Describe 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).

Nothing to report.

What was the impact on other disciplines?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

Nothing to report.

What was the impact on technology transfer?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to report.

What was the impact on society beyond science and technology?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to report.

- 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:

Changes in approach and reasons for change

Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

Nothing to report.

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.

Timeline of other DOD and NIH grants obligations in addition to the complexity of surgeries has slightly delayed our timeline. We will send a no cost extension request for the completion of animal surgeries, data collection, histology work and manuscript preparation.

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.

Nothing to report.

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

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:** 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).*

Nothing to report.

Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to report.

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.*

Nothing to report.

- **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.

Nothing to report.

- **Technologies or techniques**

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

Nothing to report.

- **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.

Nothing to report.

- **Other Products**

Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:

- *data or databases;*
- *physical collections;*
- *audio or video products;*
- *software;*
- *models;*
- *educational aids or curricula;*
- *instruments or equipment;*
- *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
- *clinical interventions;*
- *new business creation; and*
- *other.*

Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Provide the following information for: (1) 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). If information is unchanged from a previous submission, provide the name only and indicate “no change”.

Example:

Name: Mary Smith
Project Role: Graduate Student
Researcher Identifier (e.g. ORCID ID): 1234567
Nearest person month worked: 5

Contribution to Project: Ms. Smith has performed work in the area of combined error-control and constrained coding.
Funding Support: The Ford Foundation (Complete only if the funding support is provided from other than this award.)

Name: Jonathan Isaacs, M.D.
Project Role: Principal Investigator
Nearest person month worked: 0.96
Contribution to project: Regulatory process, supervising the study.
Funding support: VCU salary, MCV Physicians salary for clinical work, protocol no. ANG-CP-007,
Flow through funding from NIH: 1R34NS097113-01

Name: Satya Mallu, M.D.
Project Role: Co-Investigator
Nearest person month worked: 4.08
Contribution to project: Assisted with regulatory process, performed study surgeries.
Funding support: VCU salary, Flow-through funding from NIH: 1R34NS097113-01

Name: Gaurangkumar Patel, B.S.
Project Role: Lab technician
Nearest person month worked: 1.2
Contribution to project: Assisted with study surgeries and data analysis.
Funding support: VCU salary

Name: Alia O'Meara, M.D.
Project Role: Co-Investigator
Nearest person month worked: 0.6
Contribution to project: Breeding colony maintenance
Funding support: VCU salary and NIH Grants

Name: Dorne Yager, Ph.D.
Project Role: Co-Investigator
Nearest person month worked: 0.6
Contribution to project: Imaging
Funding support: VCU salary

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

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

Nothing to report.

What other organizations were involved as partners?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:

Organization Name:

Location of Organization: (if foreign location list country)

Partner’s contribution to the project (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner’s facilities for project activities);*
- *Collaboration (e.g., partner’s staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner’s staff use each other’s facilities, work at each other’s site); and*
- *Other.*

Nothing to report.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: N/A

QUAD CHARTS: Attached

9. APPENDICES:

1. Quad Chart

2. Preliminary Results Report as of Oct 27, 2017

3. Preliminary Results Report-Revised as of Feb 26, 2018

4. Results-Discussion as of Feb 26, 2018

Side-to-Side Supercharging Allograft

W81XWH-15-PRORP-ARA

OR150131



PI: Isaacs, Jonathan

Org: Virginia Commonwealth University

Award Amount: \$497,550

Study Aim(s)

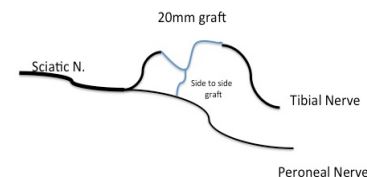
Maximize the effective length of processed acellular nerve allograft (PNA)

- Determine if side-to-side transfer will increase number and length of axons regenerating across short and long **segments** of PNA
- Determine if side-to-side transfer decreases Schwann cell senescence in PNA
- Determine the effect of side-to-side transfer on Schwann cell function in PNA
- Evaluate relative contribution of Schwann cell stimulation and “donated” axons

Approach

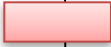
- 1) Perform 0, 1, or 3 bridge grafts to short and long rodent isograft and PNA, in normal and Thy1-GFP (fluorescent protein visible in axons) rats
- 2) Evaluate axon regeneration using imaging of Thy1-GFP rats, histology analysis, and muscle functional recovery testing
- 3) Evaluate for Schwann cell activity using immunostaining for senescence markers and nerve morpho-histology
- 4) Use retrograde labeling to identify source of axons in grafts

Schematic of side-to-side bridge grafting between peroneal nerve and 20mm graft (either isograft or PNA)



Accomplishment: ACURO approval received on Dec 01, 2016.

Timeline and Cost

Activities	Y	1 st	2 nd	3 rd	
Initial Surgeries					
In Vivo Testing, Immuohistochemistry					
Histology, Data Analysis					
Estimated Budget (\$K)		\$383K	\$114K		

Goals/Milestones

CY16 Goal – Regulatory Review Process and Breeding Colony initiation

- ✓ Regulatory Process – Received ACURO approval on Dec 01, 2016
- ✓ Thy1- GFP Rats Breeding (at very minimal level) and Colony Maintenance ongoing.

✓ All of the Thy1-GFP rats underwent initial surgeries.

CY16-17 Goals - Perform initial surgeries, testing;

- ☑ Initial Surgeries-SD rats: Ongoing
- ☐ Evaluate axon sprouting using green fluorescent protein expressing rodents: Ongoing
- ☐ Evaluate functional nerve recovery using histology and functional muscle contracture- Ongoing
- ☐ Perform retrograde labeling to evaluate axon sources
- ☐ Immunostaining for Schwann cells and senescence markers

Comments/Challenges/Issues/Concerns: N/A

Budget Expenditure to Date

Projected Expenditure: \$497,550

Actual Expenditure: \$324,157

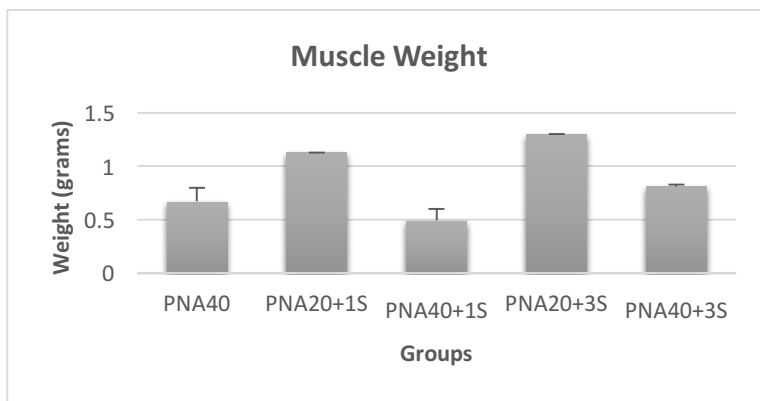
Preliminary Results

Data below is collected from 10 animals undergone nerve repair as listed below:

Group	PNA40	PNA20+1S	PNA40+1S	PNA20+3S	PNA40+3S
n	2	2	2	2	2

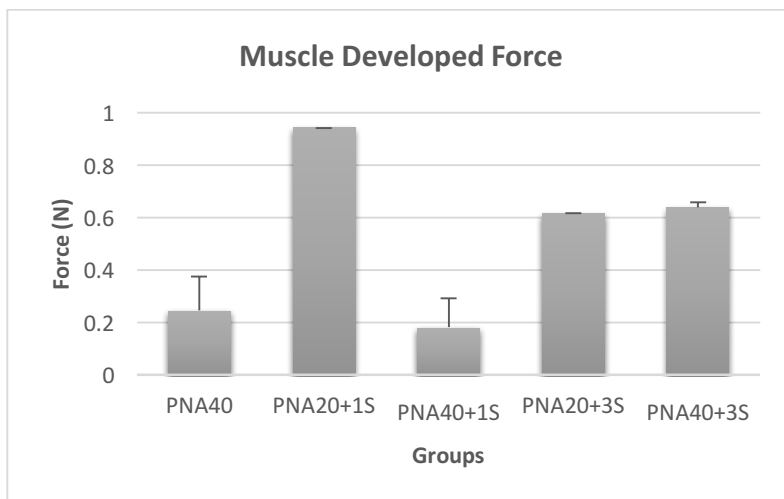
Muscle Weight (grams):

Group	PNA40	PNA20+1S	PNA40+1S	PNA20+3S	PNA40+3S
Average	0.67	1.13	0.49	1.30	0.81
ST Dev	0.13	0.00	0.11	0.00	0.02



Muscle Developed Force (N):

Group	PNA40	PNA20+1S	PNA40+1S	PNA20+3S	PNA40+3S
Average	0.245	0.942	0.182	0.616	0.639
ST Dev	0.279	0.194	0.192	0.168	0.199



No statistical analysis have been conducted as n=2 for all groups.

Preliminary Results

Data below is collected from 10 animals undergone nerve repair as listed below:

Group	n	Description
PNA20+1S	2	Repair with 20mm PNA graft and 1 bridge
PNA20+3S	2	Repair with 20mm PNA graft and 3 bridges
PNA40	2	Repair with 40mm PNA graft
PNA40+1S	2	Repair with 40mm PNA graft and 1 bridge
PNA40+3S	2	Repair with 40mm PNA graft and 3 bridges

Table 1: Experimental Groups

Muscle Weight (grams):

Group	n	Muscle Weight (grams)
PNA20+1S	2	1.13 $\pm$ 0.00
PNA20+3S	2	1.30 $\pm$ 0.00
PNA40	2	0.67 $\pm$ 0.13
PNA40+1S	2	0.49 $\pm$ 0.11
PNA40+3S	2	0.81 $\pm$ 0.02

Table 2: Muscle Weight Descriptive Statistics (value = average $\pm$ standard deviation, units = grams)

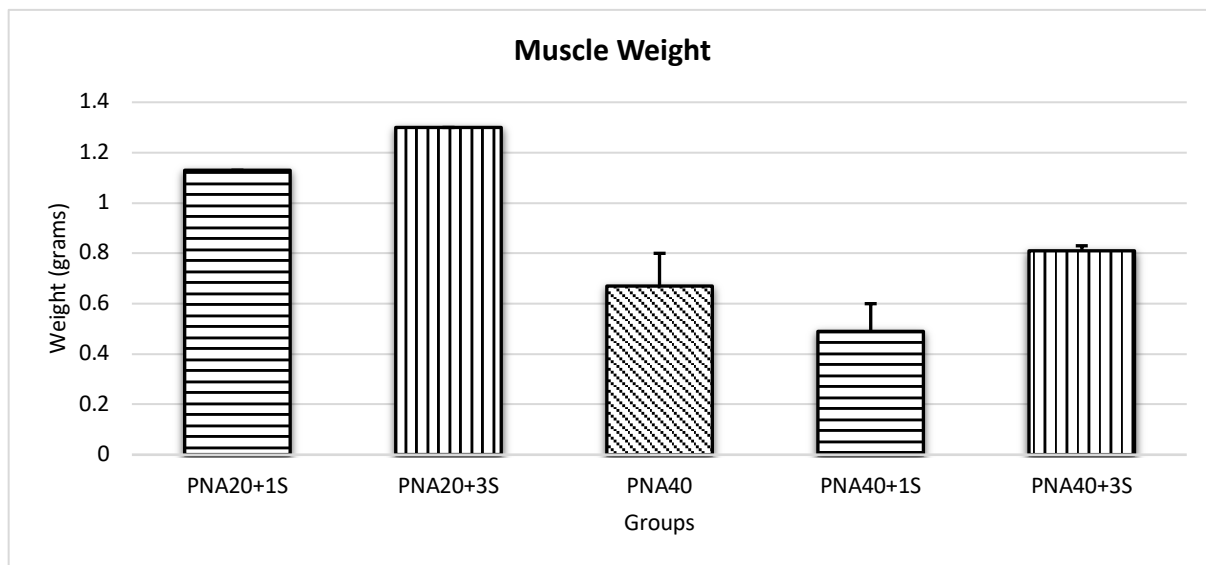


Figure 1: Muscle Weight (error bars = standard deviation)

Muscle Developed Force (Newtons (N)):

Group	n	Muscle Developed Force (N)
PNA20+1S	2	0.942 ± 0.194
PNA20+3S	2	0.616 ± 0.168
PNA40	2	0.245 ± 0.279
PNA40+1S	2	0.182 ± 0.192
PNA40+3S	2	0.639 ± 0.199

Table 3: Muscle Developed Force Descriptive Statistics (value = average $\pm$ standard deviation, units = Newtons (N))

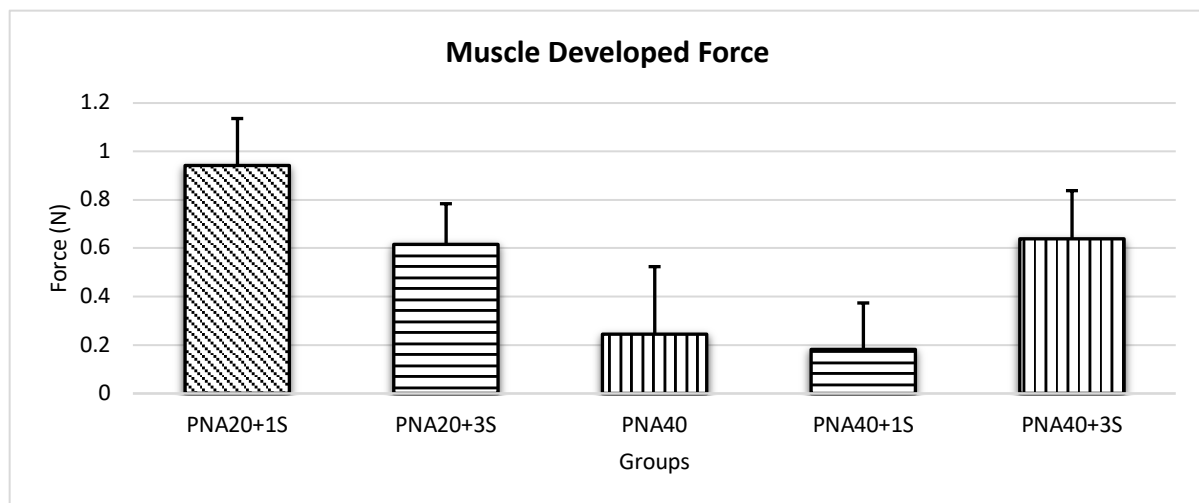


Figure 2: Muscle Developed Force (error bars = standard deviation)

No statistical analyses have been conducted as $n=2$ for all groups.

Results - Discussion

Definitive conclusions cannot be drawn as the majority of animals are still in the pre-testing phase. The data collected is from 10 animals (n=2, 5 groups):

- Muscle mass seems to increase as number of supercharging sites increase.
- In 40mm acellular nerve allograft, supercharging with one bridge did not positively affect muscle recovery. Supercharging with three bridges seems to positively affect the nerve regeneration across a 40mm allograft. This is not surprising since Ladek and all showed that one side-to-side bridge graft was not effective but three side-to-side bridge grafts were effective in improving nerve regeneration in a chronically denervated distal nerve stump (Neurosurgery 2011).
- A greater treatment effect seems likely in the 40mm allograft group compared with the 20mm allograft group. This would actually support our hypothesis. Untreated acellular nerve allograft can support nerve regeneration at 20mm implying a minimal neurotrophic deficit at that length. Adding axons via side-to-side supercharging is meant to improve the neurotrophic environment in the acellular nerve allograft. If the environment is not compromised, there is not much to improve. By contrast, we know that at 40mm lengths, the Schwann cells that have migrated into the nerve allograft seem to become less active and enter a senescent state. The improved nerve regeneration seen following side-to-side supercharging in the 40mm allografts implies that the Schwann cell function is preserved. Further data including retrograde labeling to identify the source of the regenerating axons and immunohistology staining for senescence markers is still pending.