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14. ABSTRACT

Side-to-side bridge grafting into chronically denervated nerve tissue has been shown to enhance the neurotrophic environment and increase axon regeneration and may be useful in processed acellular nerve allograft (PNA). Rodent tibial nerves were repaired with 20mm or 40mm isograft or PNA with 0, 1 or 3 side-to-side bridge grafts. Testing at 20 weeks consisted of muscle and nerve assessment including neuron back-labeling. Average recovered force production with 40mm PNA+3 bridge grafts was greater than with untreated 40mm PNA. Schwann cell counts were higher at the bridge points in the PNA. A greater proportion of senescent cells were detected in the distal aspect of the untreated 40mm PNA compared to all other 40mm grafts. Retrograde labeling demonstrated only between 6.6% and 17.7% of the reinnervating neurons were derived from the peroneal nerve pool. Side-to-side grafting seemed to improve the neurotrophic environment in long PNA.

15. SUBJECT TERMS
Supercharging of Nerves, Isograft, Processed Acellular Nerve Allograft, Bridge Graft

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1. INTRODUCTION:

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:

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))	100%
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))	100%
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) 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.)	100%
	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)	100%
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)	100%
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)	100%
	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)	100%
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)	100%
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)	100%
	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)	100%
2	Nerve Samples from Batch 2 (prepare, section, stain, image, and analyze)	100%
3	Nerve Samples from Batch 3 (prepare, section, stain, image, and analyze)	100%
	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)	100%
2	Nerve Samples from Batch 2 (prepare, section, stain, image, and analyze)	100%
3	Nerve Samples from Batch 3 (prepare, section, stain, image, and analyze)	100%
	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)	100%
2	Nerve Samples from Batch 2 (prepare, section, stain, image, and analyze)	100%
3	Nerve Samples from Batch 3 (prepare, section, stain, image, and analyze)	100%
	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	100%
2	Spinal Cord Samples (collect, prepare, section, image, and analyze)	100%
	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	100%
2	Manuscript preparations	100%
	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.

Major Accomplishments:

- The effects of 1 or 3 side-to-side bridge grafts on functional axon regeneration across processed nerve allograft (PNA) was assessed
    - Side-to-side bridge grafting in a rodent model was shown to positively impact functional recovery across long PNA. Possible physiological mechanisms include hyper-innervation, Schwann cell amplification, and neurotrophic enhancement.

Specific Accomplishments:

- 84 Sprague-Dawley rats underwent excision of 10mm of tibial nerve and immediate repair with 20 or 40mm PNA or isograft (ISO) (Figure 1)
    - A standard biceps femoris-semitendinosis muscle splitting approach was used to expose the right sciatic nerve and major branches. The tibial nerve was transected half way between the sciatic division and the distal part of the tibial nerve at the level of the knee and 5mm of nerve was excised from either side of the transection.
    - Zero, 1, or 3 side-to-side bridge grafts were created between intact donor peroneal nerves and PNA (but not ISO) (Figure 2 and 3).
        - Eighteen Sprague-Dawley rats underwent immediate repair with 20mm (n=9) or 40mm (n=9) PNA.
        - Eighteen Sprague-Dawley rats underwent immediate repair with 20mm (n=9) or 40mm (n=9) ISO.
        - Forty-eight Sprague Dawley rats underwent immediate repair with 20mm (n=24) or 40mm (n=24) PNA combined with 1 (n=12 per 20mm or 40mm group) or 3 (n=12 per 20mm or 40mm group) side-to-side bridging nerve transfer(s) from the peroneal nerve.
            - All bridging side-to-side nerve grafts were performed using 6mm rodent PNA sutured to perineurial windows (500μm in diameter) into the side of the peroneal nerve and the graft material.
            - The grafts were positioned in a "C" or serpentine configuration into a subcutaneous pocket in the ipsilateral thigh so side-to-side bridge graft insertion points could be brought near the intact donor nerve. All nerve coaptations were performed with 3 to 4 10-0 nylon sutures under surgical microscope magnification.
- Donor isograft nerves were harvested from Sprague-Dawley rats.
    - Bilateral sciatic nerves were exposed as described above using aseptic technique. The bilateral sciatic and tibial nerves were resected and the peroneal nerve portion of the sciatic nerve stripped away to maintain uniform diameter and the graft was trimmed down to 20mm length. Two 20mm long isografts were sutured together using 10-0 nylon for 40mm long grafts. The grafts were immediately sutured into the recipient nerve.
- Processed acellular rodent nerve allograft was obtained from AxoGen, Inc. (Alachua, FL) based on the same process in which commercially available human acellular nerve allograft is prepared. A proprietary detergent process removed cells and cellular debris from the endoneurial channels. An enzymatic process removed chondroitin sulfate proteoglycans from the endoneurial tubes and gamma irradiation sterilized the processed nerves before being sterilely packaged and shipped to our lab.
- Muscle strength was tested at 20 weeks post implantation.

The tibial nerve was exposed and the gastrocnemius muscle and tendon isolated. The hind limb was secured to a platform via femoral condyle and distal tibial Kirschner wires. Gastrocnemius tendons were transected and coupled to a MLT500/A force transducer (AD Instruments, Inc.,

- Colorado Springs, CO) using 4-0 silk suture. A Grass stimulator (Model SD9, Astro-Med Inc., West Warwick, RI) with platinum electrodes was used apply a 2ms duration and 2ms delay stimulation to the proximal tibial nerve. Muscle fiber length was optimized using the Blix curve.20 Three supramaximal stimulations (5V, 1Hz) were delivered to the sciatic nerve with 2-minute rest intervals between stimulations. Contraction strength was converted to digital data using ADI Instruments Power Lab system (ADInstruments, Inc., Colorado Springs, CO) and recorded using a Sony VAIO laptop (Sony Corporation, Tokyo, Japan). For groups with side-to-side bridge grafts, testing was performed after peroneal nerve transection to minimize donor axon contribution.
- Gastrocnemius muscles were harvested and weighed.
  - The 20mm repair constructs were significantly different with regards to muscle weight (Figure 4, $F(3,31)=3.652$, $p=0.023$), but not force (Figure 4, $F(3,32)=0.454$, $p=0.716$). Repair with 20mm ISO resulted in larger muscles when compared to PNA20 and PNA20+1S ($p=0.011$ and $p=0.015$, respectively). There was also a trend toward larger muscles when repaired with PNA20+3S than repair with either PNA20 or PNA20+1S ($p=0.074$ and $p=0.057$, respectively).
  - There were significant differences between the 40mm repair constructs for both muscle weight (Figure 4, $F(3,32)=6.995$, $p=0.001$) and force (Figure 4, $F(3,31)=5.340$, $p=0.004$). Repair with ISO40 resulted in significantly larger muscles than all PNA groups ($p<0.01$ for all comparisons). Repair with ISO40 also resulted in greater force output compared to PNA40 and PNA40+1S. ($p=0.002$ for both comparisons). There was a trend toward greater force output in the PNA40+3S group compared to the PNA40 and PNA40+1S groups ($p=0.064$ and $p=0.052$, respectively).
  - Conclusions:
    - Functional recovery is achieved across 20mm PNA and ISO in a rodent model
    - Functional recovery is superior across 40mm ISO compared with PNA in a rodent model
    - Functional recovery was (most likely) improved with three (but not one) side-to-side bridge grafts

- Nerve specimens were harvested at 20 weeks post implantation.
  - The grafts and tibial nerves distal and proximal to the graft were harvested en bloc and fixed in 4% paraformaldehyde at 4°C. Nerve sections were obtained 5mm distal and 5mm proximal to the graft and stained with toluidine blue for axon counts, inner diameters, and g-ratio measurements. Histologic specimens underwent histomorphologic measurements on 9 predetermined high powered fields (40X) and were multiplied by the appropriate factor as determined by the cross sectional area of the specimen using a 10X image.
  - There were no significant differences between groups for proximal measures of nerve histology (Table 1, axon inner diameters, g-ratios, or axon counts) or distal axon counts in 20mm groups. There were significant differences in distal nerve inner diameters ($F(3,28)=3.562$, $p=0.027$) and g-ratios ($F(3,28)=5.234$, $p=0.005$) for 20mm groups. Repair with ISO20 resulted in larger inner diameters when compared to all groups ($p<0.02$ for all comparisons) and larger g-ratios when compared to PNA20 and PNA20+3S ($p<0.004$ for both comparisons). There was a trend towards larger g-ratios in the ISO20 group when compared to PNA20+1S ($p=0.059$).

- Conclusions:
  - Axon maturation and myelination (but not necessarily regeneration) is superior is 20mm ISO compared with PNA
- There were no significant differences between groups for proximal or distal measures of nerve histology (Table 1, axon inner diameters, g-ratios, or axon counts) in the 40mm groups. Within each treatment group, measures of distal nerve histology (inner diameters, g-ratios, and axon counts) were consistently smaller/lower when compared to measures of proximal nerve histology within the same graft ($p<0.05$ for 21 of the 24 group comparisons).
  - Conclusions:
    - Axon regeneration, maturation, and myelination may not be different between ISO and PNA (though this observation may be underpowered due to larger than expected variability)

- Nerve specimens underwent immunostaining with S100 for Schwann cells. Immunostaining was performed at 25%, 50%, and 75% marks along the length of the graft in all groups which corresponded to the location of side-to-side bridge grafts (PNA20+1S and PNA 40+1S: 50% mark and PNA20+3S and PNA40+3S: 25%, 50% and 75% mark).
  - There were significant differences between the 20mm repair groups for absolute Schwann cell counts (Table 2 and Figure 5) at the 50% ($F(3,16)=8.970$, $p=0.001$) and 75% ($F(3,16)=15.181$, $p<0.001$) mark on the grafts. At the 50% mark, both PNA20+1S and PNA20+3S had significantly higher Schwann cell counts than ISO20 and PNA20 groups ($p<0.02$ for all comparisons). At the 75% mark, PNA20+3S resulted in significantly more Schwann cells than all other groups ($p<0.001$ for all comparisons).
  - There were also significant differences between the 40mm repair groups for absolute Schwann cell counts (Table 2 and Figure 6) at the 50% ($F(3,16)=8.361$, $p=0.001$) and 75% ($F(3,16)=7.228$, $p=0.003$) mark on the grafts. At the 50% mark, both PNA40+1S and PNA40+3S groups had significantly higher Schwann cell counts than the ISO40 and PNA40 groups ($p<0.05$ for all comparisons). At the 75% mark, repair with PNA40+3S resulted in more Schwann cells than the ISO40 and PNA40 groups ($p=0.001$ for both comparisons) and PNA40+1S resulted in significantly more Schwann cells than the PNA40 group ($p=0.045$).
  - Conclusions:
    - Schwann cell populations are augmented by side-to-side bridge grafting
    - Unclear source of Schwann cells (from donor nerve across bridge grafts verses augmented in situ population)
- Nerve specimens underwent immunostaining with β-galactosidase (β-gal) for the quantification of senescence (Senescence Detection Kit, Abcam, Cambridge, MA) was performed according to manufacturer's protocol. Immunostaining was performed at 25%, 50%, and 75% marks along the length of the graft in all groups which corresponded to the location of side-to-side bridge grafts (PNA20+1S and PNA 40+1S: 50% mark and PNA20+3S and PNA40+3S: 25%, 50% and 75% mark).

There were significant differences between the 20mm repair groups for the number of cells in senescence (Table 2) at the 25% ($F(3,20)=7.899$, $p=0.001$) and 50% ($F(3,20)=5.905$, $p=0.005$) mark on the grafts. At both the 25% and 50% mark, ISO20 group had significantly more cells in senescence than all PNA groups ($p<0.004$ for all comparisons). In the 40mm groups, there were only significant differences in the number of senescent cells at the 50% mark of the graft ($F(3,17)=6.217$, $p=0.001$) with the ISO40 group having less senescent cells than all other groups (Table 2, $p<0.01$ for all comparisons).

- Conclusions:
  - Senescence highest in middle and distal aspects of long PNA
  - Senescence is still pronounced in ISO
- A third variable (percentage of Schwann Cells in Senescence) was calculated as follows: $\left(\frac{\beta-gal}{S100}\right) * 100$.
  - There were significant differences between the 20mm repair groups for the percentage of cells in senescence at all 3 locations along the length of the graft (Table 2, 25%: $F(3,12)=4.111$, $p=0.032$; 50%: $F(3,12)=9.076$, $p=0.002$; 75%: $F(3,12)=10.349$, $p=0.001$). At the 25% mark, repair with ISO20 resulted in a greater proportion of cells in senescence when compared to PNA20+1S ($p=0.005$). At the 50% and 75% mark of the grafts, repair with ISO20 resulted in a greater proportion of cells in senescence than all PNA groups ($p<0.03$ for all comparisons).
  - There were significant differences between the 40mm repair groups at the 50% and 75% mark of the grafts (Table 2, 50%: $F(3,12)=8.283$, $p=0.003$; 75%: $F(3,10)=11.626$, $p=0.001$). At the 50% mark, repair with PNA40 resulted in significantly more cells in senescence than all other groups ($p<0.02$ for all comparisons). There was a trend towards more cells in senescence with PNA40+3S when compared to ISO40 group at the midpoint of the graft ($p=0.056$). Similarly, at the 75% mark, repair with PNA40 resulted in more cells in senescence than all other groups ($p<0.001$ for all comparisons).
  - Conclusions:
    - Percentage of Schwann cells within PNA in senescence is decreased by side-to-side bridge grafting

- Retrograde labeling was performed at 20 weeks post implantation (3 rats in each side-to-side bridge graft group)
  - After inducing isoflurane anesthesia, the peroneal and tibial nerves were exposed and crushed 5mm distal to the most distal graft site. The proximal stumps were surrounded by wells of vacuum grease formed on small squares of Parafilm, and soaked in tap water for 5 minutes. The water was removed and replaced with dextran amine crystals (10,000 MW, fixable) conjugated to AlexaFluor 488 or AlexaFluor 594 (Invitrogen, Carlsbad, CA). Crystals were then hydrated with a drop of saline. Tracers were left in place for 90 minutes and then the entire surgical field was irrigated three times with normal saline. Distal tips of the nerve were separated and coated with 6µm of fibrin glue. Wounds were closed and rats recovered.
  - Five days post labeling, the rats were euthanization with Euthasol (150mg/kg) and perfused transcardially with saline followed by 4% paraformaldehyde or periodate-lysate-paraformaldehyde fixative. The L2–S1 segments of spinal cord were removed and stored overnight in 20% sucrose solution at 4°C for cryoprotection. Serial transverse sections were cut at 20µm on a cryostat and mounted directly onto slides. Sections were cover-slipped and viewed with optics optimized for epifluorescence examination of Alexafluor 488 and Alexafluor 594. The retrogradely labeled motoneurons were identified, counted and included for descriptive purposes.
    - The percentage of peroneal neurons (out of all peroneal neurons) that donated branches through 1 or 3 bridge grafts ranged from 26.2% in the PNA40+3S group to 34.7% in the PNA20+3S group.

        - These peroneal neurons that donated across the bridge grafts represented as few as 6.6% of the total neuron pool supplying the distal tibial nerve in the PNA40+3S group and up to 17.7% of the total neuron pool supplying the distal tibial nerve in the PNA20+3S group.
    - Conclusions:
        - Unlikely that functional improvement with side-to-side bridge grafting due to augmented muscle innervation with donor axons
        - More donor axons appear to enter the shorter PNA than longer PNA

Additional aspects of study that did not provide meaningful information;

- Transgenic fluorescent protein (Thy1-green fluorescent protein) expressing rats were included in the study. GFP rats have been genetically modified so that the axons appear fluorescent green (under a fluorescence-enabled microscope). Expected visualization of elongated axons into isograft or PNA was meant to provide qualitative data demonstrating donor axon ingrowth.

    - Sixteen *Thy1*-GFP Sprague-Dawley rats underwent transection (and excision of 10mm of nerve) and repair of a unilateral tibial nerve with 20 or 40mm processed nerve allograft (PNA) or isograft. Zero, 1, or 3 side-to-side bridge grafts were performed between the intact donor peroneal nerve and the isograft or PNA.

- 4 *Thy1*-GFP rats underwent immediate repair of the transected nerve with 20mm (n=2) or 40mm (n=2) isograft.

- 4 *Thy1*-GFP rats underwent immediate repair of the transected nerve with 20mm (n=2) or 40mm (n=2) processed acellular nerve allograft.

- 8 *Thy1*-GFP rats underwent immediate repair of the transected nerve with 20mm (n=4) or 40mm (n=4) processed acellular nerve allograft combined with one (n=2) or three (n=2) side-to-side bridging nerve transfer(s) from the peroneal nerve. The bridging side-to-side nerve grafts were 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 were 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 were brought near the intact donor nerve. All nerve coaptations were performed with three to four 10-0 nylon stitches under surgical microscope magnification.
- At 10 weeks, general anesthesia was again induced, and the right sciatic nerve and graft were exposed. A fluorescence-enabled microscope utilizing GFP (488 nm) fluorescent and bright field filters were used to image the grafts. Images were digitalized and recorded. Although magnification, exposure time, brightness, and contrast were all standardized, images suffered from insufficient fluorescence. Axons were not visualized. The rats were euthanized.
- Conclusions:

    A literature search did not provide insight regarding the failure of this aspect of our study protocol. However, discussion with other investigators familiar with this genetically modified strain suggested that the genetic modification had been lost within our breeding colony.

Proximal	**Inner Diameters (*um*)**	**G-ratios**	**Axon Count**	*Distal*	**Inner Diameters (*um*)**	**G-ratios**	**Axon Count**	*Proximal vs. Distal*	**Inner Diameters p-value**	**G-ratios p-value**	**Axon Count p-value**
ISO 20	6.88 (0.75)	0.66 (0.03)	6716 (1172)	**ISO 20**	3.34 (0.29)	0.52 (0.06)	4311 (1422)	**ISO 20**	0.008*	0.053	0.062
PNA 20	5.53 (1.40)	0.57 (0.09)	8234 (428)	**PNA 20**	2.76 (0.44)	0.42 (0.07)	2642 (1002)	**PNA 20**	0.052	0.022*	0.006*
PNA 20 + 1S	5.68 (1.02)	0.60 (0.07)	6931 (2309)	**PNA 20 + 1S**	2.77 (0.75)	0.46 (0.07)	3288 (2165)	**PNA 20 + 1S**	0.001*	0.005*	0.043*
PNA 20 + 3S	5.79 (0.98)	0.60 (0.06)	6633 (1284)	**PNA 20 + 3S**	2.72 (0.21)	0.42 (0.04)	2805 (802)	**PNA 20 + 3S**	<0.001*	0.001*	<0.001*
ISO 40	6.12 (1.39)	0.65 (0.07)	6953 (2186)	**ISO 40**	2.55 (0.51)	0.40 (0.07)	2135 (1444)	**ISO 40**	0.001*	<0.001*	0.025*
PNA 40	5.90 (0.79)	0.62 (0.04)	6928 (1227)	**PNA 40**	1.61 (0.61)	0.29 (0.06)	564 (344)	**PNA 40**	0.001*	0.001*	<0.001*
PNA 40 + 1S	5.88 (0.58)	0.61 (0.03)	7041 (1995)	**PNA 40 + 1S**	2.26 (2.14)	0.31 (0.16)	1813 (2347)	**PNA 40 + 1S**	<0.001*	<0.001*	0.003*
PNA 40 + 3S	5.18 (1.54)	0.55 (0.10)	5361 (1574)	**PNA 40 + 3S**	2.08 (0.71)	0.32 (0.07)	809 (472)	**PNA 40 + 3S**	0.007*	0.004*	<0.001*

Abbreviations: um=micrometers

* denotes significant differences between measures of proximal nerve histology when compared to distal nerve histology within same treatment groups at $p<0.05$.

Table 1. Nerve histology means, standard deviations (in parentheses), and statistical results for isograft (ISO), processed acellular nerve allograft (PNA) repair alone, PNA with 1 side-to-side bridge graft (PNA + 1S), or PNA with 3 side-to-side bridge grafts (PNA + 3S) for 20mm and 40mm repair lengths.

Absolute Schwann Cell Quantification (S100)	**S100 (25%)**	**S100 (50%)**	**S100 (75%)**	*Quantification of Schwann Cells in Senescence (β-gal)*	**β-gal (25%)**	**β-gal (50%)**	**β-gal (75%)**	*Percentage of Schwann Cells in Senescence (Bgal/S100)*100*	**Percentage in Senescence (25%)**	**Percentage in Senescence (50%)**	**Percentage in Senescence (75%)**
ISO 20	0.38 (0.16)	0.24 (0.08)	0.13 (0.11)	**ISO 20**	0.17 (0.05)	0.15 (0.03)	0.13 (0.05)	**ISO 20**	51.0 (23.7)	67.6 (20.5)	125.2 (52.1)
PNA 20	0.38 (0.09)	0.31 (0.13)	0.23 (0.10)	**PNA 20**	0.10 (0.04)	0.10 (0.04)	0.09 (0.03)	**PNA 20**	33.9 (5.9)	37.5 (26.8)	42.3 (15.9)
PNA 20 + 1S	0.36 (0.07)	0.51 (0.10)	0.23 (0.01)	**PNA 20 + 1S**	0.07 (0.02)	0.09 (0.02)	0.09 (0.02)	**PNA 20 + 1S**	19.0 (6.2)	18.4 (7.5)	38.6 (7.0)
PNA 20 + 3S	0.42 (0.06)	0.46 (0.06)	0.45 (0.06)	**PNA 20 + 3S**	0.10 (0.03)	0.09 (0.02)	0.09 (0.03)	**PNA 20 + 3S**	30.4 (5.9)	17.2 (1.1)	17.0 (0.4)
ISO 40	0.35 (0.05)	0.31 (0.03)	0.20 (0.04)	**ISO 40**	0.11 (0.05)	0.11 (0.03)	0.13 (0.04)	**ISO 40**	33.2 (22.5)	31.4 (10.9)	51.3 (16.3)
PNA 40	0.32 (0.04)	0.28 (0.05)	0.19 (0.04)	**PNA 40**	0.09 (0.03)	0.16 (0.03)	0.20 (0.06)	**PNA 40**	33.6 (14.3)	63.3 (8.3)	106.5 (23.4)
PNA 40 + 1S	0.37 (0.07)	0.39 (0.06)	0.30 (0.13)	**PNA 40 + 1S**	0.07 (0.03)	0.15 (0.02)	0.14 (0.04)	**PNA 40 + 1S**	21.6 (7.4)	38.9 (8.8)	38.9 (9.2)
PNA 40 + 3S	0.42 (0.11)	0.42 (0.07)	0.40 (0.08)	**PNA 40 + 3S**	0.08 (0.03)	0.17 (0.02)	0.20 (0.09)	**PNA 40 + 3S**	22.0 (9.0)	44.3 (5.8)	49.2 (11.5)

Abbreviations: S100=Schwann cell stain, β-gal=Senescence marker

Table 2. Means, standard deviations (in parentheses), and statistical results for absolute Schwann cell and total cell scenescence quantification at 25%, 50%, and 75% of isograft (ISO), processed acellular nerve allograft (PNA), PNA with 1 side-to-side bridge graft (PNA + 1S), or PNA with 3 side-to-side bridge grafts (PNA + 3S) for 20mm and 40mm repair lengths. *percentages calculated only when S100 and β-gal data available from the same specimen (does not represent the quotient of means).

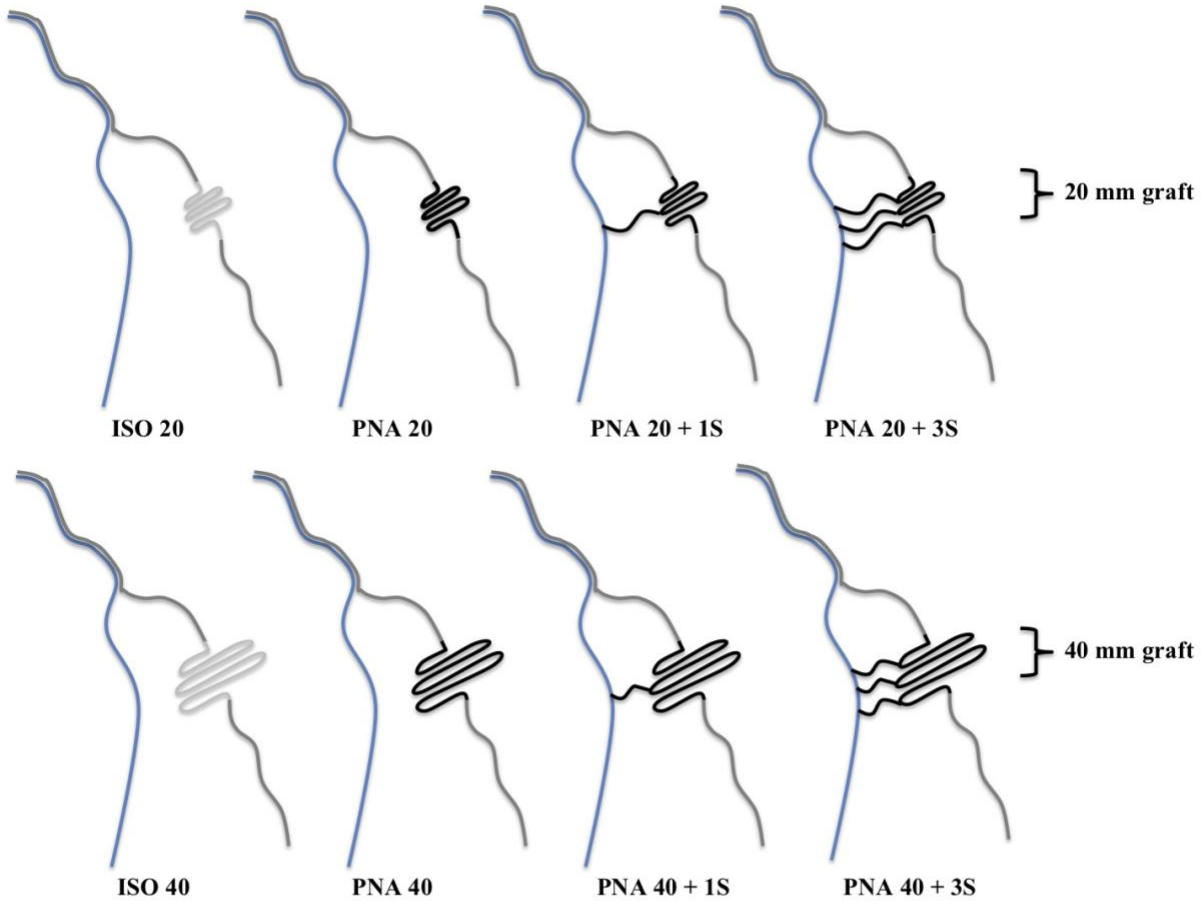


Figure 1. Schematic representation of 10mm tibial nerve (grey line) resection and repair with 20mm (top row) or 40mm (bottom row) isograft (ISO) (left column), processed acellular nerve allograft (PNA) alone (left middle column), PNA + 1 side-to-side bridge graft (1S, right middle column), or PNA + 3 side-to-side bridge grafts (3S, right column). Combined blue and grey line represents the sciatic nerve, the blue line represents the peroneal nerve, the grey line represents the tibial nerve, the light grey line represents ISO and the black lines represent the PNA and any associated bridge grafts from the peroneal nerve.

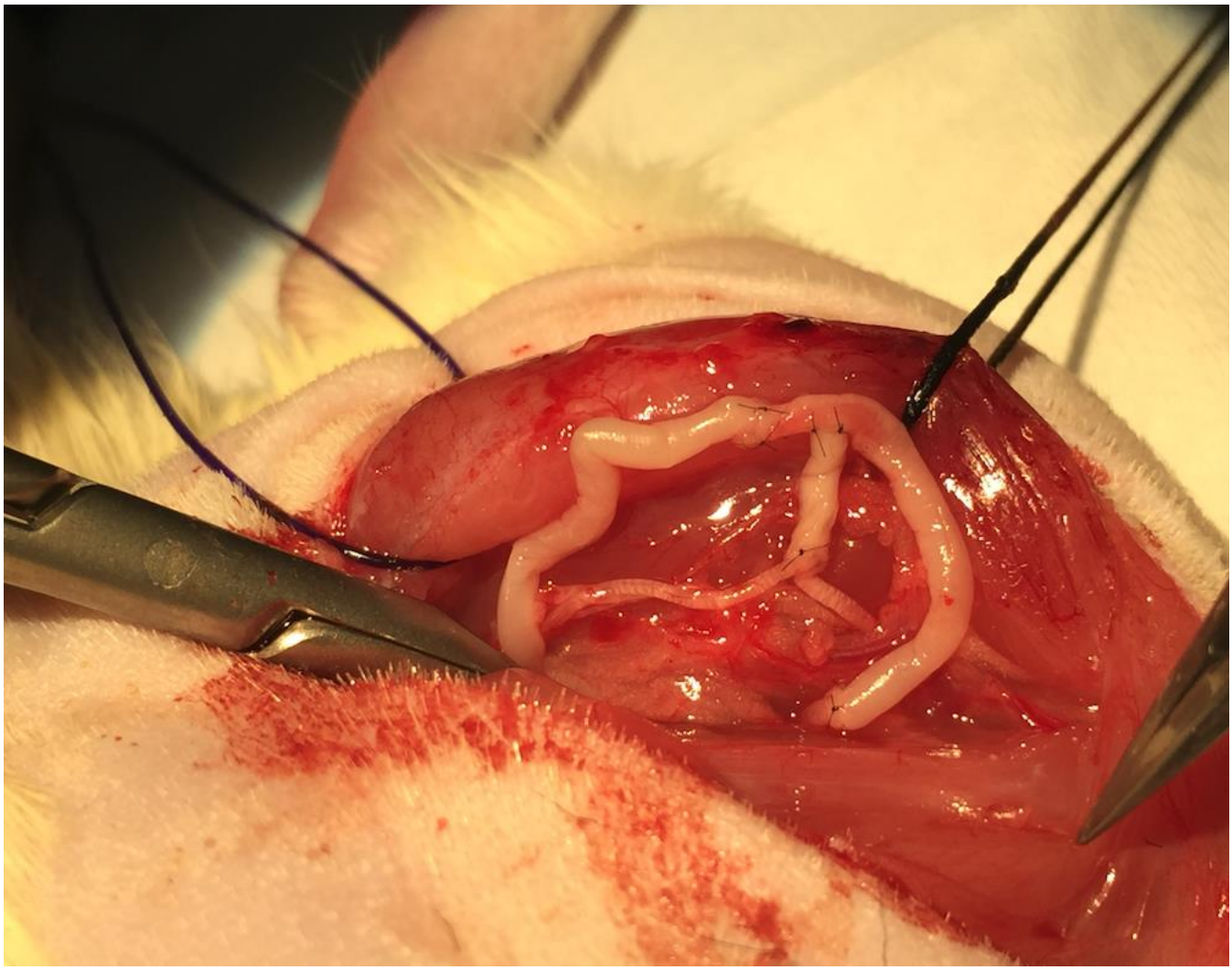


Figure 2. Representative example of 40mm processed acellular nerve allograft with 1 side-to-side bridge graft.

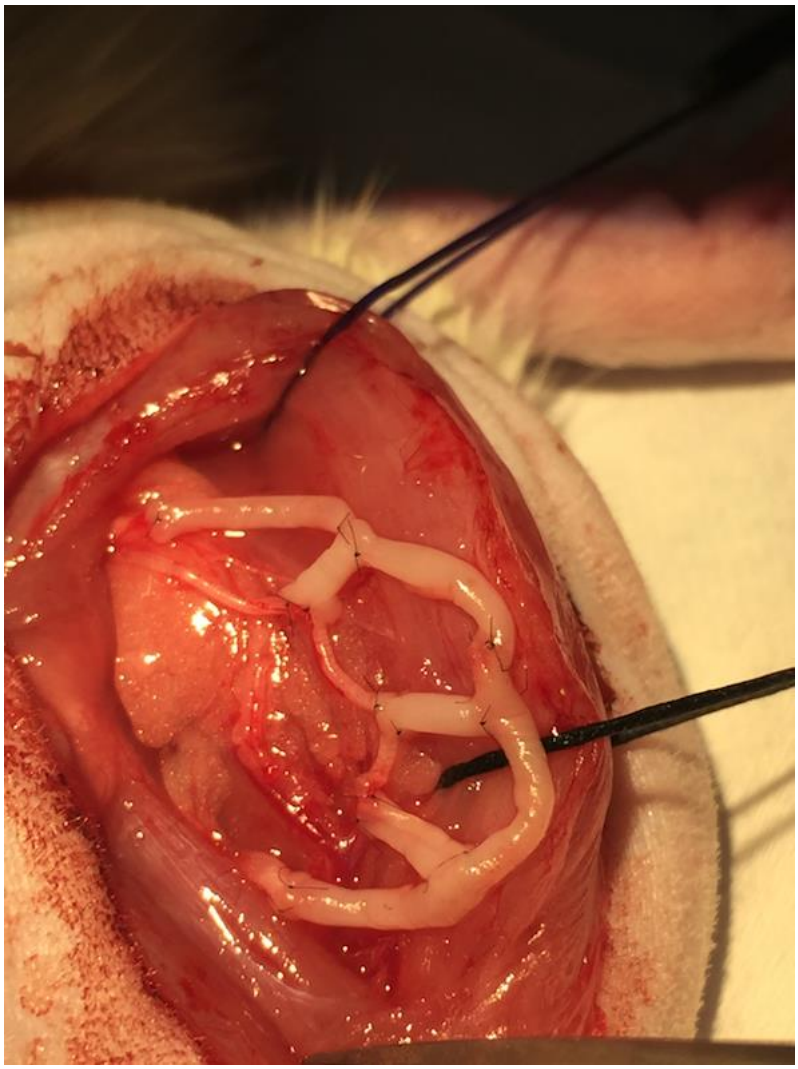


Figure 3. Representative example of 40mm processed acellular nerve allograft with 3 side-to-side bridge grafts.

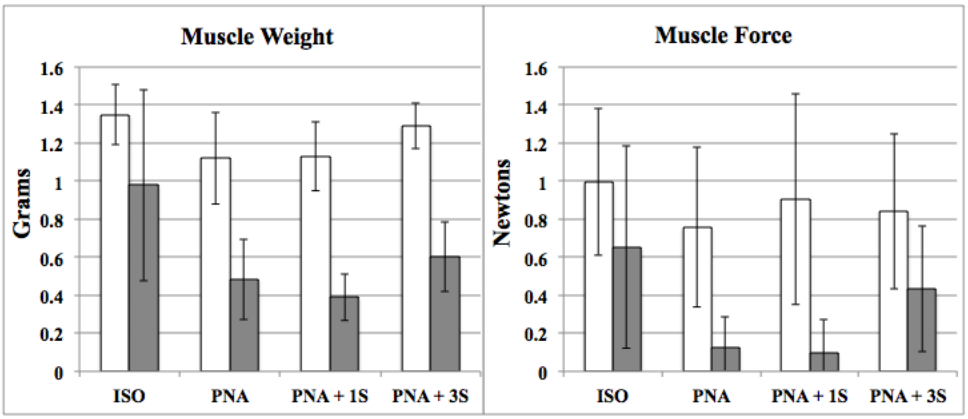


Figure 4. Means and standard deviations (error bars) for muscle weight (left) and muscle force (right) for 20mm (white bars) and 40mm (grey bars) repair constructs with either isograft (ISO), processed acellular nerve allograft (PNA) alone, PNA + 1 side-to-side bridge graft (1S), or PNA + 3 side-to-side bridge grafts (3S).

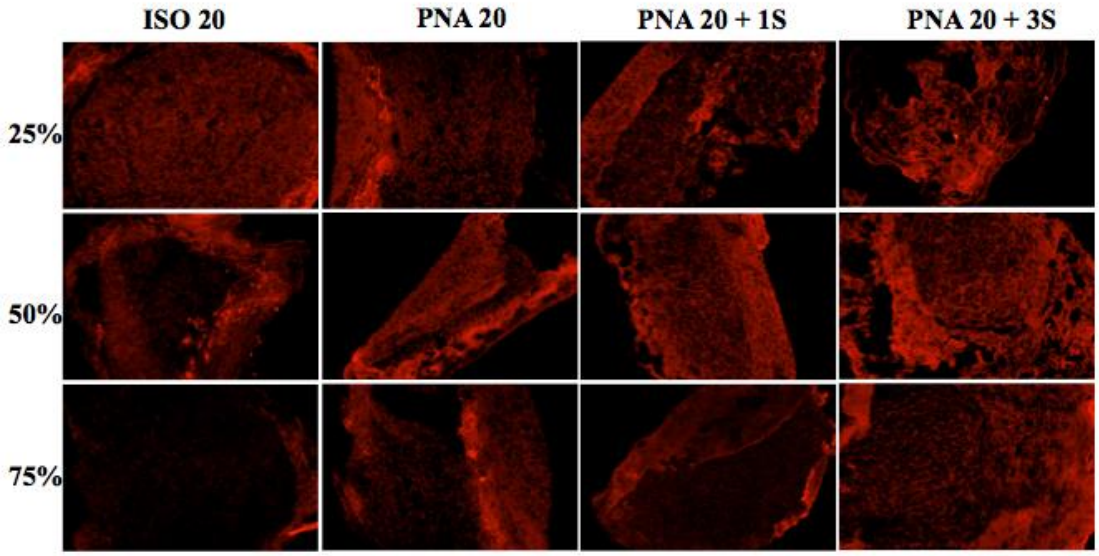


Figure 5. Representative images for immunostaining with S100 for Schwann cells for 20mm repair constructs. Top row represents the 25% mark on the graft, middle row represents 50% mark on the graft, and bottom row represents 75% mark on the graft. For PNA the 20 +1S group, the side-to-side bridge graft was at the 50% mark. For the PNA 20 + 3S group, the side-to-side bridge grafts were at the 25%, 50%, and 75% mark.

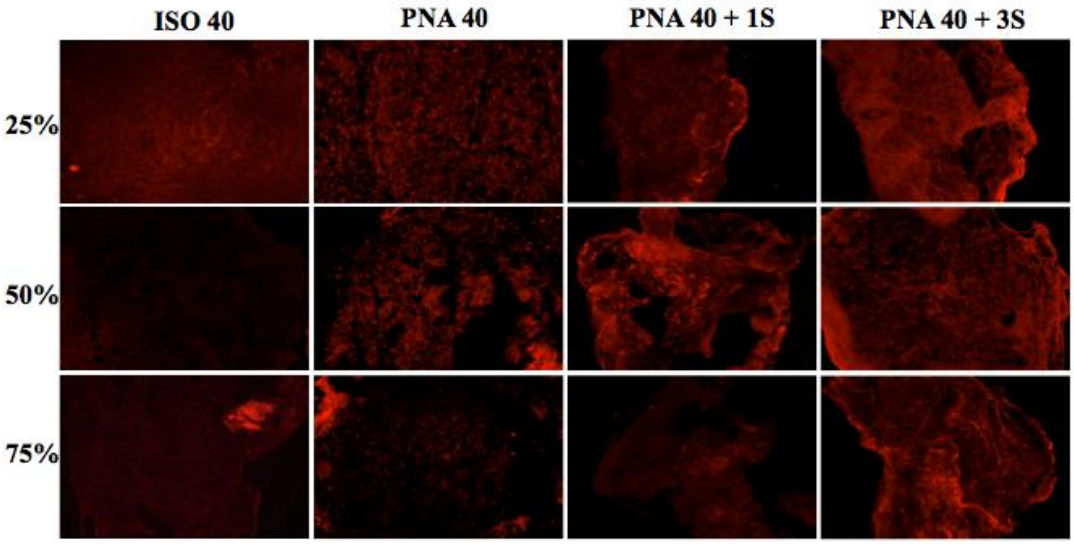


Figure 6. Representative images for immunostaining with S100 for Schwann cells for 40mm repair constructs. Top row represents the 25% mark on the graft, middle row represents 50% mark on the graft, and bottom row represents 75% mark on the graft. For PNA the 40 +1S group, the side-to-side bridge graft was at the 50% mark. For the PNA 40 + 3S group, the side-to-side bridge grafts were at the 25%, 50%, and 75% mark.

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.

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.

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.

Nothing to report.

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).

Our study supports previous proposed theories regarding supercharging and Schwann cell activity. The bridge grafts provided a pathway for both donor axons and Schwann cells to enter the nerve allograft. Schwann cell senescence seemed to decrease.

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:*

Thy1-GFP animals underwent in-vivo imaging of nerves using fluorescence-enabled microscope utilizing GFP (488nm) fluorescent and bright field filters. Images were analyzed to quantify the amount of regeneration occurred in the graft and the source of the regeneration; fluorescence intensity was suboptimal and images did not provide meaningful data. This information is not included in final report.

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 sent a no cost extension request and it was approved thru March 29, 2019 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).*

Side-to-Side Supercharging Nerve Allograft Enhances Neurotrophic Potential Jonathan Isaacs, Mark A. Feger, Satya Mallu, Gaurangkumar Patel, Monika Debkowska, Dorne Yager, Brady Ernst, Sravya Chilukuri, Matthew Moser Camden Kurtz MUSCLE and NERVE (Submitted).

  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.*

1. Side-to-side Supercharging Allograft
Jonathan Isaacs, Mark A. Feger, Satya Mallu, Gaurangkumar Patel,
Monika Debkowska, Dorne Yager, Brady Ernst, Sravya Chilukuri, Matthew Moser
Camden Kurtz
Military Health System Research Symposium (MHSRS) August 2019 (Accepted)

2. Side-to-Side Bridge Grafting Enhances Axon Regeneration in Long Acellular Nerve Allograft
Jonathan Isaacs, Mark Feger, Satya Mallu, Dorne Yager, Sravya Chilukuri, Brady Ernst
74th Annual Meeting of the American Society for Surgery of Hand (ASSH), September 5-7, 2019, Las Vegas. (Accepted).

3. Side-to-Side Supercharging Improves Functional Recovery Following Long Acellular Nerve Allograft Repair in a Rat Model
Jonathan Isaacs, MD, Satya Mallu, MD, Gaurangkumar Patel, B.S. and Monika Debkowska, MD
Podium presentation at the AAHS Annual Meeting, January 30 - February 2, 2019, at the JW Marriott Hotel in Palm Desert, CA.

- **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: 1.5
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, Axogen, Inc., Polyganics.

Name: Satya Mallu, M.D.
Project Role: Co-Investigator
Nearest person month worked: 8
Contribution to project: Assisted with regulatory process, performed study surgeries, Data Anaysis.
Funding support: VCU salary, Flow-through funding from NIH: 1R34NS097113-01, Axogen, Inc.,

Name: Gaurangkumar Patel, B.S.
Project Role: Lab technician
Nearest person month worked: 8
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: 2
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: 1
Contribution to project: Imaging, Histology
Funding support: VCU salary

Name: Mark Feger, PhD
Project Role: Medical Student
Nearest person month worked average per annum: 0.5
Contribution to project: Data Analyses, Assisting in Manuscript preparation.
Funding support: None.

Name: Brady Ernst
Project Role: Medical Student
Nearest person month worked average per annum: 2
Contribution to project: Retrograde Labeling Histology, Data Analysis
Funding support: None.

Name: Matt Moser
Project Role: Medical Student
Nearest person month worked average per annum: 2
Contribution to project: Retrograde Labeling Histology, Data Analysis
Funding support: None.

Name: Sravya Chilukuri
Project Role: Medical Student
Nearest person month worked average per annum: 2
Contribution to project: Schwann cell Senescence Histology, Data Analysis
Funding support: None.

Name: Camden Kurtz, M.S.
Project Role: Lab technician
Nearest person month worked: 1.5
Contribution to project: Nerve Histomorphometry
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:

QUAD CHARTS:

9. APPENDICES

1. Quad Chart

Side-to-Side Supercharging Allograft

W81XWH-16-1-0662
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

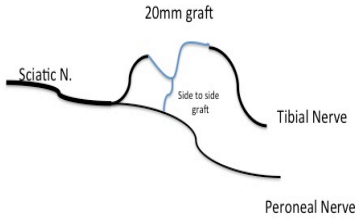
•Determine the effect of side-to-side transfer on Schwann cell function in PNA

•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)



Timeline and Cost

Activities	Y	1st	2nd	3rd	
Initial Surgeries					
Implantation in Rodents for reaction					
In Vivo Testing					
Histology, Data Analysis					
Estimated Budget ($K)		**$383K**	**$114K**		

Goals/Milestones

CY16-17 Goals – Regulatory Review Process and Breeding Colony initiation

✔ Regulatory Process – Received ACURO approval on Dec 01, 2016
✔ Thy1- GFP Rats Breeding and Colony Maintenance.
✔ All of the Thy1-GFP rats underwent initial surgeries.

CY17-18 Goals - Perform initial surgeries, testing;

✔ Initial Surgeries and Terminal Surgeries on Sprague Dawley Rats.
✔ Evaluate functional nerve recovery using histology and functional muscle contracture
✔ 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: $497,550

Richmond, VA. Updated: **07/24/2019**