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TITLE: Reinnervation of Paralyzed Limb muscle by Nerve-Muscle-Endplate Grafting Technique

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14. ABSTRACT

The goal of this research is to evaluate the efficacy of our new surgical technique called nerve-muscle-endplate band grafting (NMEG) for limb reinnervation in a rat model. Immediate transection of the nerve innervating the tibialis anterior (TA) muscle, a NMEG pedicle was harvested from the ipsilateral lateral belly of the gastrocnemius muscle (GM) and implanted to the native motor zone (NMZ) of the TA muscle for reinnervation. Three months after surgery, functional recovery of the TA treated with the NMEG-NMZ technique was evaluated by muscle force measurement. The extent of axonal regeneration in the target muscle was assessed using immunohistochemical techniques. Our results showed that NMEG-NMZ technique resulted in better functional recovery (81% of the control) as compared with commonly used nerve end-to-end anastomosis (EEA) (50%). The NMEG-NMZ induced extensive axonal regeneration in the treated muscle. These findings suggest that NMEG-NMZ technique is effective for limb reinnervation.

15. SUBJECT TERMS Nerve-muscle-endplate band grafting, peripheral nerve injury, limb reinnervation, axonal regeneration, motor endplate band, native motor zone, functional recovery

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1. INTRODUCTION:

Traumatic peripheral nerve injury (PNI) to the extremities and resultant muscle paralysis represent a significant cause of morbidity and disability in both military and civilian populations. Although a number of surgical procedures have been used to restore motor function following PNIs, the currently available surgical procedures result in poor functional recovery. Poor motor recovery after PNIs and nerve repair is due primarily to insufficient axonal regeneration and failure to reinnervate the denervated motor endplates (MEPs) in the target muscle. In response to this, we developed a novel surgical technique called nerve-muscle-endplate grafting (NMEG) technique for muscle reinnervation. The underlying concept is that a paralyzed muscle could be reinnervated by transplanting an NMEG pedicle from a neighboring donor muscle. A MEP band with a nerve branch and terminals that innervates an expendable muscle can be transplanted to a more functionally important denervated muscle to restore its motor function. Using a rat neck muscle model, we performed a series of studies to investigate the NMEG technique. As MEP reinnervation is critical for motor recovery, we modified the procedures by implanting the NMEG pedicle to the native motor zone (NMZ, which contains a MEP band) of the target muscle. This NMEG-NMZ is based on the rationale that denervated MEPs in the NMZ are preferential sites for reinnervation. Unlike other nerve repair methods, NMEG-NMZ provides an abundant source of nerve terminals that favor axonal regeneration and facilitates rapid axon-MEP connections. We have demonstrated that NMEG-NMZ results in excellent functional recovery in a neck muscle model. This research is to evaluate the efficacy of the NMEG-NMZ technique for limb reinnervation.

2. KEYWORDS:

Nerve-muscle-endplate band grafting (NMEG), native motor zone (NMZ), peripheral nerve injury, limb reinnervation, axonal regeneration, axon-endplate connection, functional recovery

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Aim 1: Evaluate the efficacy of NMEG-NMZ for *immediate* reinnervation of denervated limb muscle, and determine the beneficial effects of nerve growth-stimulating methods (i.e., intraoperative brief ES and local administration of the ENFs NGF and FGF-2) on axonal regeneration and outcomes of NMEG-NMZ.

Aim 2: Evaluate the efficacy of NMEG-NMZ for reinnervation of *chronically* denervated limb muscle, and assess the potential of specific therapeutic strategies (i.e., sensory protection and intramuscular injection of ENFs) for preservation of muscle mass and MEPs prior to NMEG-NMZ.

This research was designed to test our hypothesis that NMEG-NMZ technique will be effective for reinnervation of denervated limb muscle, and that the outcomes of NMEG-NMZ will be augmented by incorporating specific therapeutic strategies that enhance nerve regeneration as well as preserve muscle mass and MEPs in the target muscle. For NMEG-NMZ, we harvested an NMEG pedicle from lateral belly of the gastrocnemius muscle (GM) to reinnervate the experimentally denervated ipsilateral tibialis anterior (TA) muscle. Adjunctive therapies used in this research include intraoperative brief electrical stimulation (ES) and local application of exogenous neurotrophic factors (ENF). For comparison, we use nerve end-to-end anastomosis (EEA) and autologous nerve graft (ANG) as technique controls. We also use 3-month and 6-month denervation as controls.

In this research, two reinnervation models (immediate and delayed) are used. Immediate reinnervation is performed immediately after TA nerve transection, while delayed reinnervation is carried out at the end of 3 months after TA nerve transection. The animals are randomly assigned to 12 groups (15 rats/each group), 6 immediate and 6 delayed reinnervation groups.

Immediate (Imm) reinnervation groups: (1) Imm-NMEG-NMZ (Imm-NN); (2) Imm-NN/ES; (3) Imm-NN/ENF; (4) Imm-EEA; (5) Imm-ANG; and (6) 3-month denervation.

Delyed (Del) reinnervation groups: (1) Del-NN; (2) Del-ENF/NN; (3) Del-sensory protection/NN (SP/NN); (4) Del-EEA; (5) Del-ANG; and (6) 6-month denervation.

The animals undergo post-operative evaluations after a 3-month recovery period. The post-operative evaluations include functional assessment (i.e., muscle force measurement and static toe spread analysis), neural studies (i.e., axonal regeneration, motor endplates, and axon-endplate connections), and muscle studies (i.e., muscle mass, fiber size, fiber type and myosin heavy chain composition). The results from this research will provide evidence for the effectiveness of the NMEG-NMZ and adjunctive therapies for limb reinnervation.

What was accomplished under these goals?

• **Major Activities**

We performed the following major activities over the past year.

Animal surgeries

We performed 180 operations on 90 animals (2 operations for each rat) in 6 immediate reinnervation groups (15 rats/per group) that include: (1) Imm-NN; (2) Imm-NN/ENF; (3) Imm-NN/ES; (4) Imm-EEA; (5) Imm-ANG; and (6) 3-month denervation. All the surgical procedures were successfully performed.

Muscle force measurements

All animals in the immediate reinnervation groups, except for 3-month denervation group, were subjected to muscle force measurement (n = 75 rats) 3 months after surgery to evaluate functional recovery. The force data from animals in Imm-NN and Imm-EEA have been analyzed and the results are summarized below (see Key Outcomes). The force data from the animals in Imm-NN/ENF, Imm-NN/ES, and Imm-ANG groups have been collected and will be analyzed in the following year.

Tissue studies

At the end of experiments, the left experimental TA and right control for each rat were removed, measured, and prepared for tissue studies. The tissue samples were sectioned and and stained using histological and immunohistochemical techniques. The results from the animals in Imm-NN, Imm-EEA, and 3-month denervation groups have been collected and summarized below. The muscle samples from other groups will be processed and analyzed in the following year.

• **Specific Objectives**

Data from the animals in the Imm-NN, Imm-EEA, and 3-month denervation groups have been analyzed to determine the differences in the outcomes between both techniques.

Objective 1: To determine the degree of functional recovery of the paralyzed muscles reinnervated by either NMEG-NMZ technique or EEA.

Objective 2: To determine the extent of axonal regeneration and axon-MEP connections in the muscles reinnervated by NMEG-NMZ and EEA procedures.

Objective 3: To document morphological and histological changes (i.e., muscle mass, fiber size, and fiber type and myosin heavy chain composition) in the NMEG-NMZ and EEA reinnervated muscles.

• **Major Procedures**

Data from the rats in Imm-NN and Imm-EEA groups have been collected, analyzed, and summarized as follows.

NMEG-NMZ surgical procedures

For Imm-NMEG-NMZ, the left TA muscle was denervated by resecting its innervating nerve. The native motor zone (NMZ) of the left TA was outlined and a muscular defect (recipient bed) was made in the NMZ of the denervated TA muscle.

We have mapped the MEP bands in the rat TA and GM muscles (**Fig. 1A**). A single MEP band is located obliquely in the middle of the TA. The GM is composed of two neuromuscular compartments, lateral (GM-l) and medial (GM-m), each of which has a vertically positioned MEP band. The TA is innervated by the deep peroneal nerve, which divides into two to three branches immediately after entering the upper portion of the muscle. These branches further give off

numerous nerve terminals to supply MEPs (**Fig. 1B**). The GM-l and GM-m are innervated by two separate nerve branches derived from the tibial nerve. Both nerve branches enter the top of the muscle, travel in the superior and inferior directions from the origin to insertion, and give off terminals to innervate MEPs (**Fig. 1B**). These data provide a guide for NMEG-NMZ.

The NMZs in the left TA and GM were outlined according to the locations of the MEP band as visualized by acetylcholinesterase staining (**Fig. 1A**), motor point, and muscular nerve branches (**Fig. 1B**) as revealed by Sihler's stain. The left TA was then denervated by excising a 10-mm segment of its nerve (**Fig. 1C**). Specifically, the peroneal nerve will be cut proximally, 5 mm away from the ischiatic trifurcation (i.e., starting point of the tibial, common peroneal, and sural nerves), and distally, 10 mm away from the first transection. Both ends of the nerve were ligated to prevent nerve regeneration. A muscular defect, with the same dimensions as the NMEG pedicle, was made in the NMZ of the left denervated TA. An NMEG pedicle containing a block of muscle (~10×5×3 mm), axon terminals, and a MEP band with neuromuscular junctions was harvested from the NMZ of the GM-l in continuity with its nerve branch. Finally, the NMEG was embedded into the TA defect and sutured with 10-0 nylon (**Fig. 1C**).

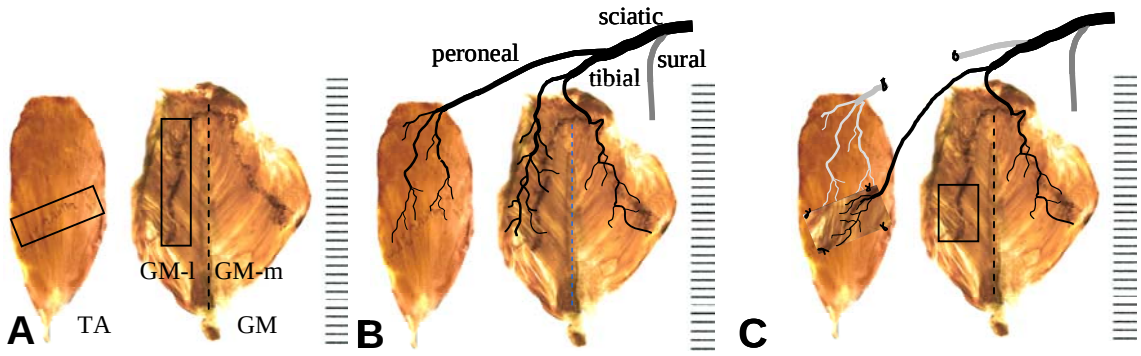


Fig. 1. (**A**) NMZs in the rat left TA and GM muscles. The MEP bands (boxed regions) with numerous neuromuscular junctions (black dots) in the TA and GM are visualized by whole-mount acetylcholinesterase staining. Note that each of the GM-l and GM-m compartments in the GM has its own MEP band. The vertical dished line in the GM indicates midline. (**B**) Schematic of nerve supply patterns of the TA and GM as demonstrated by Sihler's stain, showing three major nerve branches (i.e., peroneal, tibial and sural) of the sciatic nerve. Note that GM-l and GM-m are innervated by separate nerve branches derived from the tibial nerve. (**C**) NMEG-NMZ transplantation, showing that an NMEG pedicle with a nerve branch is harvested from the GM-l (boxed region) and implanted to the NMZ of the denervated TA muscle.

The experimentally denervated TA muscle in the Imm-NN, Imm-NN/ES, and Imm-NN/ENF groups was immediately reinnervated with NMEG-NMZ technique (**Fig. 2**). Surgical procedures were successfully performed for each rat.

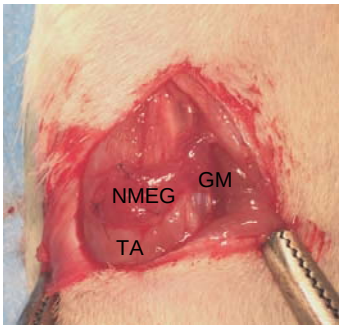


Fig. 2. A photograph, showing implantation of a nerve-muscle-endplate graft (NMEG) from the gastrocnemius muscle (GM) to the denervated tibialis anterior (TA) muscle on the left side for muscle reinnervation in a rat model.

NMEG-NMZ/ENF procedures

Animals in the Imm-NN/ENF group underwent focal administration of ENFs (a mixture of NGF and FGF-2) to investigate their ability to enhance axonal regeneration and outcomes of NMEG-NMZ. During NMEG-NMZ surgery, the muscular defect created on the TA was covered with 0.2 ml of fibrin sealant (TISSEEL Kit®; Baxter) containing recombinant rat NGF and FGF-2 (R&D Systems, 100 ng/ml and 100 µg/ml, respectively) (**Fig. 3A**). Then, the NMEG pedicle was placed on the fibrin sealant and sutured with 10-0 microsutures (**Fig. 3B**).

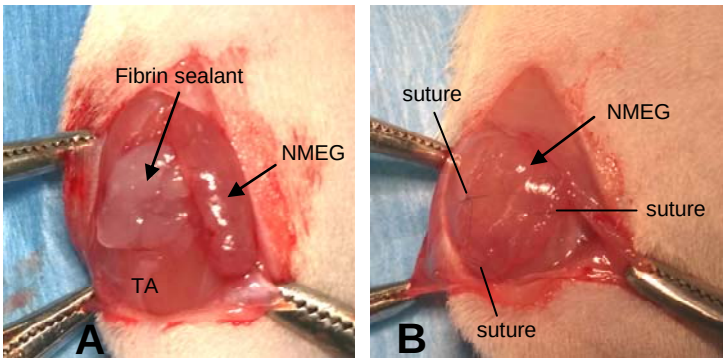


Fig. 3. NMEG-NMZ/ENF procedures. (**A**) A photograph showing the NMEG pedicle from the GM-l and profile of fibrin sealant containing NGF/FGF-2 covering on the muscular defect of the denervated TA. (**B**) A photograph after suturing the NMEG pedicle on the fibrin sealant.

ANG procedures

Animals in Imm-ANG group were subjected to ANG procedure (technique control). A nerve gap defect was made by resecting a 10-mm segment of the TA nerve 5 mm proximal to the motor point. The gap defect was bridged with the same length of sural nerve (sensory) using 10-0 nylon microsutures (**Fig. 4**).

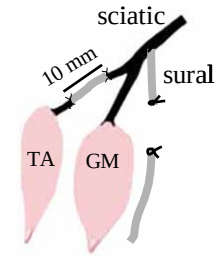


Fig. 4. Schematic of ANG procedure. Note that a 10-mm segment of the TA nerve gap defect is bridged with the same length of sural nerve.

Maximal muscle force measurement

Three months after surgery, maximal muscle force was measured on both TA muscles for each rat. Specifically, the distal tendon of the TA was severed, tied with 5-0 silk suture, and connected to a servomotor lever arm with a force transducer. The nerve branch reinnervated the left TA and the right intact TA nerve were stimulated. Isometric contractions of the TA were obtained with 200 ms trains of biphasic rectangular pulses. The duration of each phase of stimulation pulse was set at 0.2 ms and train frequency was set at 200 pulses/s. The stimulation current was gradually increased until the tetanic force reached a plateau (**Fig. 5**).

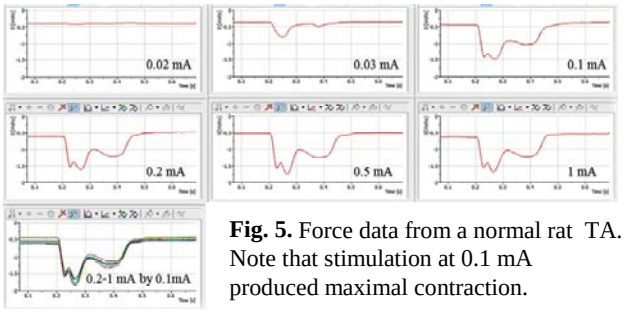


Fig. 5. Force data from a normal rat TA. Note that stimulation at 0.1 mA produced maximal contraction.

• **Key Outcomes**

NMEG-NMZ resulted in excellent functional recovery

Muscle force measurement: Muscle force measurement showed that NMEG-NMZ resulted in excellent functional recovery. The percentage of functional recovery of the reinnervated TA muscle was determined as compared with that of the contralateral control muscle in each rat. At 3 months after surgery, the mean muscle force of the NMEG-NMZ reinnervated TA was measured to be 81% of the control **(Fig. 6**), whereas that of the EEA reinnervated muscles was 50% of the control (data not shown). These findings demonstrated that the NMEG-NMZ technique resulted in better muscle force recovery as compared with the commonly used EEA nerve repair.

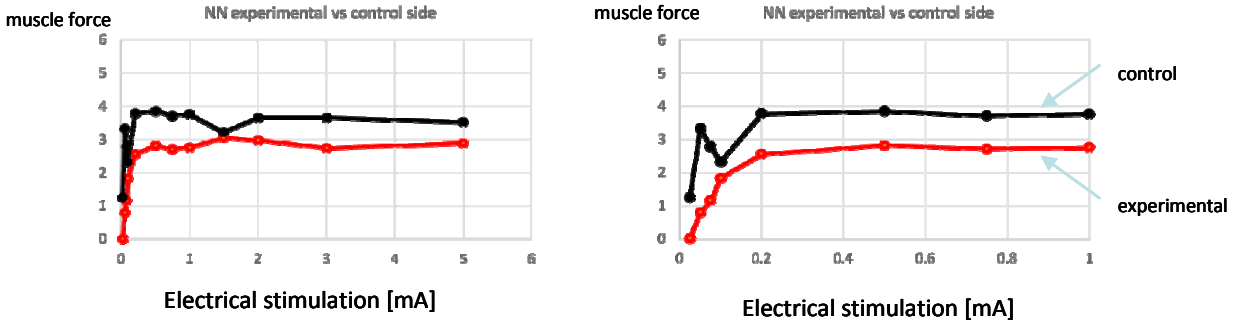


Fig. 6. Mean muscle force from operated and control TA muscles of the rats in the Imm-NN group. The passive tension was set at a moderate level (0.08 N). Stimulation was made with a 0.2-second train of 0.2-millisecond-wide biphasic pulse at frequency of 200 Hz. Operated TA muscle with implanted NMEG pedicle (shown in red) when compared to control muscle on the opposite side (shown in black) has larger stimulation threshold, reaches the level of maximal force with larger stimulation current and has smaller maximal force. Maximal muscle force was calculated as the average muscle force to stimulation currents from 0.5-1 mA. Average maximal muscle force level on the operated side (0.865 N) was 80% of muscle force on the control side (1.060 N).

Static toe spread analysis: We used static toe spread analysis to assess limb function after surgery. The rat was placed in an acrylic 40×20×20 cm container on a transparent base plate. The footprints were clearly seen on the plantar view. A digital camera was positioned underneath the base plate to photograph the plantar surface of the rat hind limb paws. Our data showed that NMEG-NMZ resulted in better toe spread than the EEA and denervation (**Fig. 7**).

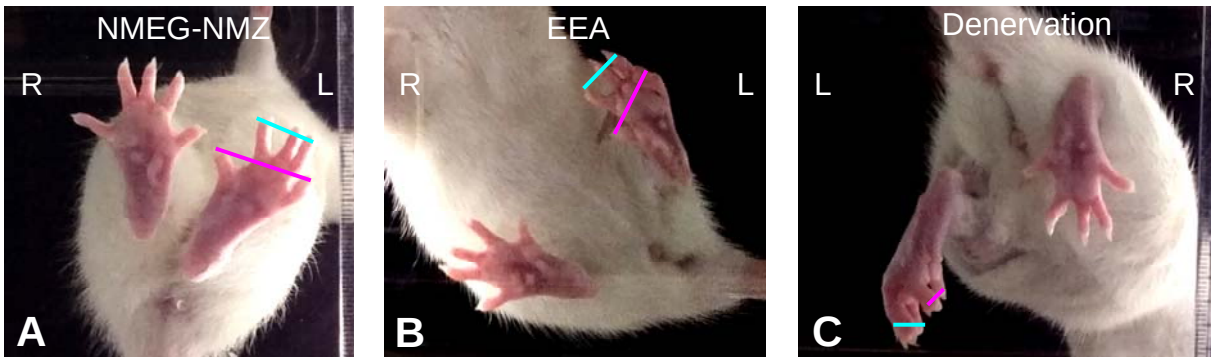


Fig. 7. Images, showing static toe spread of rats in Imm-NMEG-NMZ (**A**), Imm-EEA (**B**), and 3-month denervation (**C**) groups. The left (L) TA underwent surgical procedures, while the right (R) TA served as control for each rat. Note that distances between 1-5 toes (purple bar) and 2-4 toes (green bar) of the left foot treated with NMEG-NMZ (**A**) were larger than those treated with EEA (**B**) and denervation control (**C**). These findings indicate that NMEG-NMZ resulted in better functional recovery as compared with EEA and denervation.

Muscle weight and myofiber morphology

At the end of experiment, both TA muscles of each rat were removed and weighed. The average muscle weight was measured to be 86% of the control for Imm-NN group (**Fig. 8A**), 71% for EEA group (**Fig. 8B**), and 26% for 3-month denervation group (**Fig. 8C**). These findings indicate that NMEG-NMZ reinnervated muscles had slight muscle atrophy. Hematoxylin and eosin-stained cross muscle sections showed that the NMEG-NMZ reinnervated TA muscles exhibited very good preservation of muscle structure and myofiber morphology with less fiber atrophy and connective tissue hyperplasia (**Fig. 8D**) as compared with the EEA reinnervated (**Fig. 8E**), 3-month denervated (**Fig. 8F**), and normal control (**Fig. 8G**) muscles.

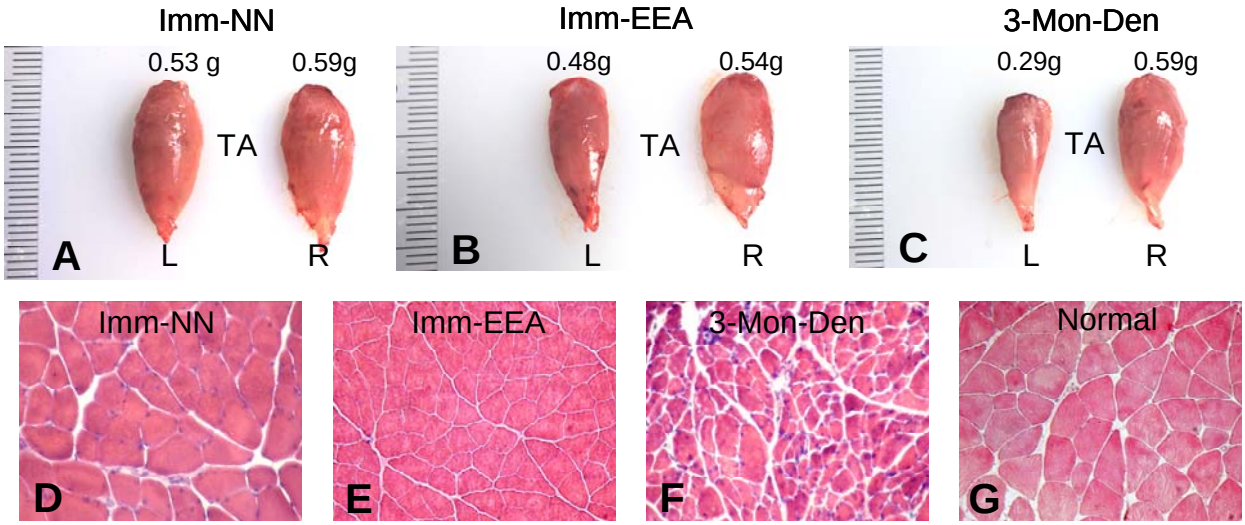


Fig. 8. Gross appearance, muscle mass, and myofiber morphology of the NMEG-NMZ reinnervated (**A**), EEA reinnervated (**B**), and 3-month denervated (**C**) left (L) treated and right (R) control TA muscles in the rats. TA muscles were removed 3 months after surgeries. Note that the mass of the left NMEG-NMZ reinnervated TA was larger than the EEA treated and 3-month denervated muscles. **D-G:** H&E stained cross sections from the TA muscles reinnervated with NMEG-NMZ (**D**), treated with EEA (**E**), 3-month denervated (**F**), and normal control (**G**). Note that the NMEG-NMZ reinnervated TA exhibited very good preservation of muscle structure with less fiber atrophy as compared with EEA treated and completely denervated muscles. The 3-month denervated TA exhibited significant myofiber atrophy. x 200 for D-G.

Nerve regeneration and muscle reinnervation

Muscle sections immunostained for neurofilament (NF) showed that NMEG-NMZ technique resulted in extensive nerve regeneration in the target muscle (**Fig. 9A**), whereas moderate level of regenerating axons was identified in the EEA reinnervated TA (**Fig. 9B**). In the NMEG-NMZ reinnervated TA, regenerating axons from the implanted NMEG supply the denervated native motor zone within the target muscle. The intramuscular axonal density was assessed by estimating the number of the NF-ir axons and the area fraction of the axons within a section area (1.0-mm^2) (**Fig. 9A', B'**). Areas with NF-positive staining were outlined, measured with public domain ImageJ software (v. 1.45s; NIH, Bethesda, Maryland). For each rat, the number and the area fraction of the NF-ir axons in the operated muscle were compared with those in the contralateral control.

Our studies showed that the mean number and area of the NF-ir axons in the NMEG-NMZ reinnervated TA muscles was computed to be 77% and 74% of the contralateral control muscles, respectively, which were larger than those of EEA treated muscles (43% and 40%, respectively). We found that NMEG-NMZ resulted in more extensive nerve regeneration and muscle reinnervation (**Fig. 9A**) as compared with EEA nerve repair (**Fig. 9B**). These findings are consistent with force data. Specifically, NMEG-NMZ technique resulted in better functional recovery (81% of the control) as compared with EEA (50% of the control).

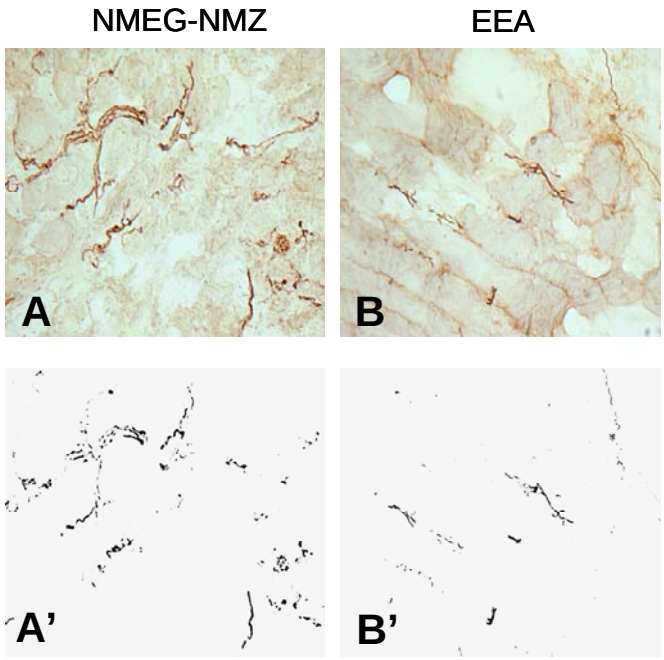


Fig. 9. Comparison of the densities of intramuscular nerve fascicles and axons between TA muscles treated with NMEG-NMZ (**A**) and EEA (**B**) three months after surgery in rats. Photomicrographs were taken from sagittal sections from the native motor zone (NMZ) in each muscle. The sections were immunostaned with antibody against neurofilament (NF) to show nerve fascicles and axons in the treated muscles. (**A'–B'**) The stained sections in A and B were opened using ImageJ software and converted to 8-bit (binary) images, color thresholded, and particle analyzed for nerve morphometry. The density of the axons was evaluated by estimating the number and area fraction of the NF-positive axons within a section area (1.0 mm^2). Note that the NMEG-NMZ reinnervated TA exhibited more extensive muscle reinnervation (mean axon count: 326; mean area: 2.68) **(A')** as compared with the TA treated with EEA (mean axon count: 169; mean area: 1.56).

Motor endplate (MEP) reinnervation

The removed TA muscle samples were cut horizontally and immunostained with double fluorescence staining to document innervated and non-innervated MEPs. In the TA muscles treated with NMEG-NMZ, the majority (86%) of the denervated MEPs were reinnervated by regenerating axons (**Fig. 10A**). Approximately 65% of the MEPs in the EEA treated TA muscles regained reinnervation (**Fig. 10B**). In the treated muscles, axonal sprouts and newly formed small MEPs were also identified. Normal (**Fig. 10C**) and denervated (**Fig. 10D**) TA muscles served as controls. In the 3-month denervated TA, only a few fragments of axons and denervated MEPs were observed.

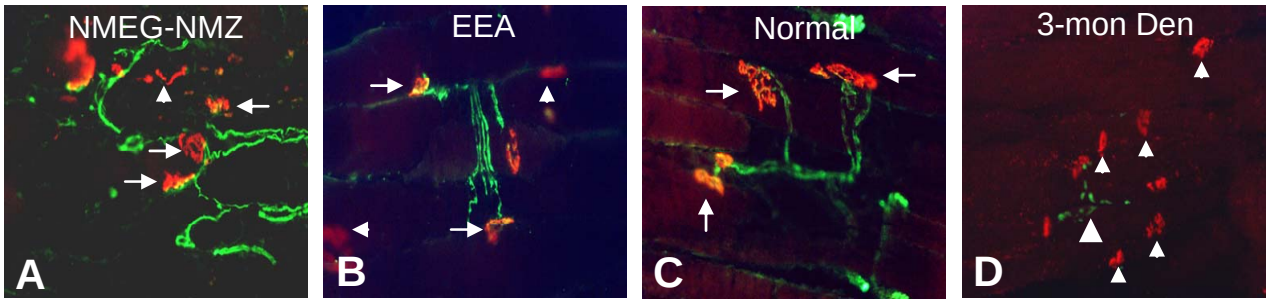


Fig. 10. Motor endplates (red) and innervating axons (green) in the TA muscles. (**A**) NMEG-NMZ reinnervated TA. (**B**) EEA treated TA. (**C**) Normal TA. Arrows in A-C indicate innervated MEPs with visible axon attachments, while arrowheads indicate noninnervated MEPs without visible axon attachments. (**D**) 3-month denervated TA. Note that only a few fragments and/or accumulations of decomposed axons (large arrowhead) and noninnervated MEPs (small arrowheads) were identified. 200x.

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

Nothing to report.

How were the results disseminated to communities of interest?

Nothing to report.

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

The following experiments will be performed during the next reporting period.

• Animals in the 6 delayed reinnervation groups (i.e., Del-NN, Del-SP/NN, Del-ENF/NN, Del-EEA, Del-ANG, and 6-month denervation) will undergo surgical and related procedures.
• We will analyze the collected force data from the TA muscles of the rats in the Imm-NN/ENF, Imm-NN/ES, and Imm-ANG groups.
• The muscle samples obtained from the animals in the Imm-NN/ENF, Imm-NN/ES, and Imm-ANG groups will be cut and stained using various histochemical and immunohistochemical techniques to assess nerve regeneration, axon-MEP connections, and fiber type-distribution.
• Submit a manuscript entitled “Limb muscle reinnervation by nerve-muscle-endplate grafting technique in a rat model”.

4. IMPACT:

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

Our studies showed that the NMEG-NMZ technique resulted in more optimal muscle reinnervation and functional recovery (81% of the control) as compared with commonly used EEA method (50%). These findings suggest that NMEG-NMZ technique is effective for limb reinnervation in a rat model. We believe that NMEG-NMZ will become a useful method in the near future to treat our patients with muscle paralysis following peripheral nerve injuries.

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?

Nothing to report.

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change

Nothing to report.

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

Nothing to report.

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

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**

Journal publications.

Nothing to report.

Books or other non-periodical, one-time publications.

Nothing to report.

Other publications, conference papers and presentations.

Nothing to report.

- **Website(s) or other Internet site(s)**

Nothing to report.

- **Technologies or techniques**

Nothing to report.

- **Inventions, patent applications, and/or licenses**

Nothing to report.

- **Other Products**

Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	Liancai Mu
Project Role:	PI
Researcher Identifier:	ORCID 0000-0002-1670-2061
Nearest person month worked:	6.2 months/per year
Contribution to Project:	Dr. Mu has performed animal surgeries as a primary surgeon, data collection and analyses.
Funding Support:	None
Name:	Jingming Chen
Project Role:	Research Associate
Researcher Identifier:	ORCID 0000-0001-7092-5728
Nearest person month worked:	6.0 months/per year
Contribution to Project:	Dr. Chen has performed all of the lab work, including lab supplies ordering, surgical preparation, animal surgery, tissue studies and data collection.
Funding Support:	None
Name:	Jing Li
Project Role:	Technician
Researcher Identifier:	ORCID 0000-0001-7968-561X
Nearest person month worked:	6.0 months/per year
Contribution to Project:	Jing Li has performed the work in the area of animal ordering, surgical preparation, animal surgery, postoperative animal care, and data collection.
Funding Support:	None
Name:	Stanislaw Sobotka
Project Role:	Co-Investigator
Researcher Identifier:	ORCID 0000-0003-0200-5078
Nearest person month worked:	2.4 months/per year
Contribution to Project:	Dr. Sobotka has performed nerve stimulation, muscle force measurements, and force data analyses.
Funding Support:	None
Name:	Themba Nyirenda
Project Role:	Biostatistician
Researcher Identifier:	ORCID 0000-0000-2128-0991
Nearest person month worked:	0.6 months/per year
Contribution to Project:	Dr. Nyirenda and the PI have discussed some issues regarding data analysis and selected statistical methods that will be used for our data analysis.
Funding Support:	None

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

Nothing to report.

What other organizations were involved as partners?

Nothing to report.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:

QUAD CHARTS:

A quad chart has been updated and submitted with attachments.

9. APPENDICES:

None.

Reinnervation of Paralyzed Limb muscle by Nerve-Muscle-Endplate Grafting Technique

W81XWH2010195

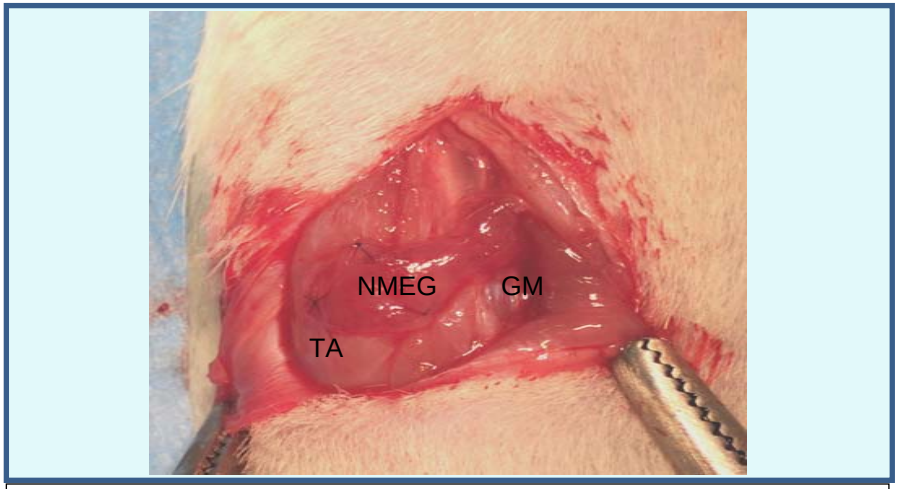
PI: Liancai Mu, MD, Ph.D **Org:** Hackensack Meridian Health - CDI **Award Amount: $1,686,264**

Study/Product Aim(s)

- **Aim 1:** Evaluate the efficacy of NMEG-NMZ technique for immediate reinnervation of denervated limb muscle, and determine the beneficial effects of nerve growth stimulation methods (i.e., brief nerve stimulation and ENF) on axonal regeneration and outcomes of NMEG-NMZ.
- **Aim 2:** Evaluate the efficacy of NMEG-NMZ technique for reinnervation of chronically denervated limb muscle, and assess the potential of specific therapies (i.e., sensory protection, and intramuscular injection of neurotrophic factors) for preservation of muscle mass and endplates prior to NMEG-NMZ surgery.

Approach

- Microsurgical procedures (NMEG-NMZ; sensory protection; nerve end-to-end anastomosis control; and denervation control).
- Physiological evaluation (muscle force measurement).
- Neural studies (nerve regeneration and axon-endplate connections).
- Muscle studies (fiber types, fiber size, muscle mass).



A photograph, showing implantation of a nerve-muscle-endplate graft (NMEG) from the gastrocnemius muscle (GM) to the denervated tibialis anterior (TA) muscle on the left side for muscle reinnervation in a rat model.

Timeline and Cost

Activities / CY	1	2	3	
Microsurgical procedures				
Physiological evaluation				
Neural studies				
Muscle studies & data analyses				
Estimated Budget ($K)	**$515k**	**$586k**	**$585k**	

Updated: (05/08/2021)

Goals/Milestones

CY1 Goal – Microsurgery and functional evaluations.

☑ Perform surgeries and some muscle force measurement.

CY2 Goals – Microsurgery, functional evaluations, and tissue studies.

☐Complete surgeries and physiological testing.

☐Perform neural and muscle studies.

CY3 Goal – Tissue studies, data collection, and data analyses.

☐ Examine intramuscular axonal regeneration and axon-endplate connections.

☐ Investigate myosin heavy chain-based fiber types, fiber size and muscle mass.

Comments/Challenges/Issues/Concerns

None.

Budget Expenditure to Date

Projected Expenditure:

Actual Expenditure: **$453,188**