AD_____

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TITLE: Strategies to Improve Chronic Nerve Regeneration and Target Reinnervation

PRINCIPAL INVESTIGATOR: Ahmet Hoke MD, PhD

RECIPIENT: Johns Hopkins University
Baltimore, MD 21218

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Fort Detrick, Maryland 21702-5012

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6. AUTHOR(S)
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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

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BALTIMORE MD 21218

8. PERFORMING ORGANIZATION REPORT NUMBER

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Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

This study was designed to examine the effects of delivery of GMF-ß to denervated nerve to preserve the "reactive" state of Schwann cells and delivery of IGF-1 to denervated muscle to preserve muscle bulk and reinnervation capacity using a model of chronic nerve injury. We were able to complete the aim focusing on IGF-1 and demonstrated that IGF-1 encapsulated into slow release nanoparticles is able to preserve muscle bulk during chronic denervation and keeps it ready for reinnervation. We did have some technical issues with the GMF-ß delivery as the commercial sources for GMF-ß were unreliable. Recently we were able to make our own GMF-ß and we are currently evaluating the effects on chronically denervated Schwann cells. As a backup we also developed a non-viral gene delivery approach. We plan to combine these two approaches in the second half of Year 2 to examine the effects on functional recovery after chronic nerve injury.

15. SUBJECT TERMS
Chronic denervation, GMF-ß, Schwann cells, IGF-1, muscle

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

TABLE OF CONTENTS

1. **INTRODUCTION:** Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.

Recovery after chronic nerve injuries is dismal due to atrophic changes that occur in denervated Schwann cell sin distal nerves and atrophy and loss of neuromuscular junctions in muscle. We aim to utilize a nanoparticle-based drug delivery platform to prevent these atrophic changes in Schwann cells and muscles using GMF-ß and IGF-1, respectively. These approaches will be evaluated individually and optimized and then tested in combination using a chronic nerve regeneration model.

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

Chronic denervation, GMF-ß, Schwann cell, IGF-1, muscle

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.

Major Task 1: To prevent atrophic changes in chronically denervated Schwann cells using GMF-ß
Major Task 2: To maintain re-innervation capacity of chronically denervated muscles using sustained delivery of IGF-1
Major Task 3: To improve functional recovery after chronic denervation by sustained local release of GMF-ß and IGF-1, delivered to the damaged nerve and target muscle, respectively

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.

During the first year of the project we accomplished the following goals. Each one is listed after the format of the SOW.

Major Task 1: To prevent atrophic changes in chronically denervated Schwann cells using GMF-ß
Task 1.1: Write animal protocol for IACUC approval
Milestone: obtain IACUC approval

Progress: This task is completed.

Task 1.2: Write animal protocol for ACURO approval
Milestone: obtain IACUC approval
Progress: This task was completed and ACURO approval was obtained on October 5, 2017

Task 1.3: To engineer the loading and examine the stability and release kinetics of GMF-ß to achieve sustained release for 12 weeks
Milestone: Optimization of release kinetics in vitro
Progress: Unfortunately, we had several technical difficulties with this task. The commercial sources of GMF-ß did not have the adequate biological activity when we tried to confirm the biological activity of GNF-ß on cultured Schwann cells to validate the lot-to-lot consistency. Since then we have examined several different commercial sources and ran into the same problem. We think this is due to the fact that most commercial sources of GMNF-ß are manufactured in bacterial cells and that secondary modifications in eukaryotic cells is needed.

In order to solve the problem, we ended up making our own GMF-ß protein using a eukaryotic expression system and tried to validate the biological activity. This strategy did nto work, but we were able to make biologically active GNF-ß using a protein refolding kit and manufacturing GMF-ß in E. coli (Figure 1). Refolded GMF-ß was able to prevent Schwann cell apoptosis induced by FBS withdrawal. We then manufactured large quantities to carry out the release kinetic optimization studies as outlined in the SOW.

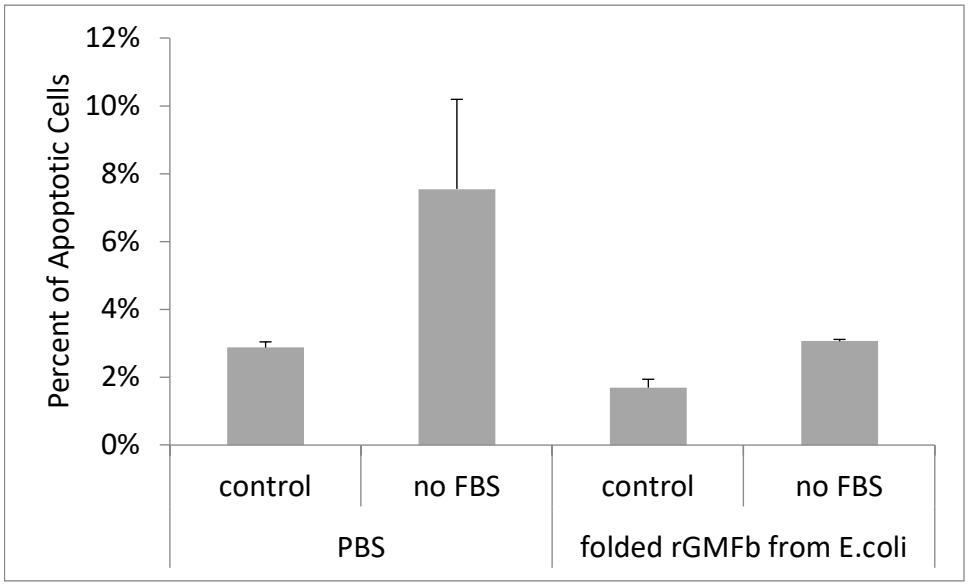


Figure 1: Rat Schwann cells were cultured in DMEM + 10% FBS. After 24 hours of incubation with 2 ug/ml rGMFb or PBS, media was changed to FBS-free DMEM that also contained rGMFb or PBS. After 20 hours, cells were fixed and labeled with Dapi. The percentage of apoptotic cells (punctate Dapi) was counted.

Unfortunately, this approach also failed. When encapsulated with the nanoparticles, the refolded GMF-ß did not retain biological activity. The GMF-ß that is released from nanoencapsulated

carrier did not retain full biological activity. We tried adjusting nanoencapsulation protocols to maintain the bioactivity of GMF-ß but failed to get reproducible results.

So we developed a backup plan to deliver GMF-ß as a plasmid and have it expressed in Schwann cells themselves as an autocrine signal. As seen in Figure 2, Schwann cells that are transfected in vitro with GMF-ß plasmid secrete GMF-ß and resist apoptotic death in response to high dose NGF or serum withdrawal. Additionally, GMF-ß transfected Schwann cells proliferate at a higher rate (Figure 3).

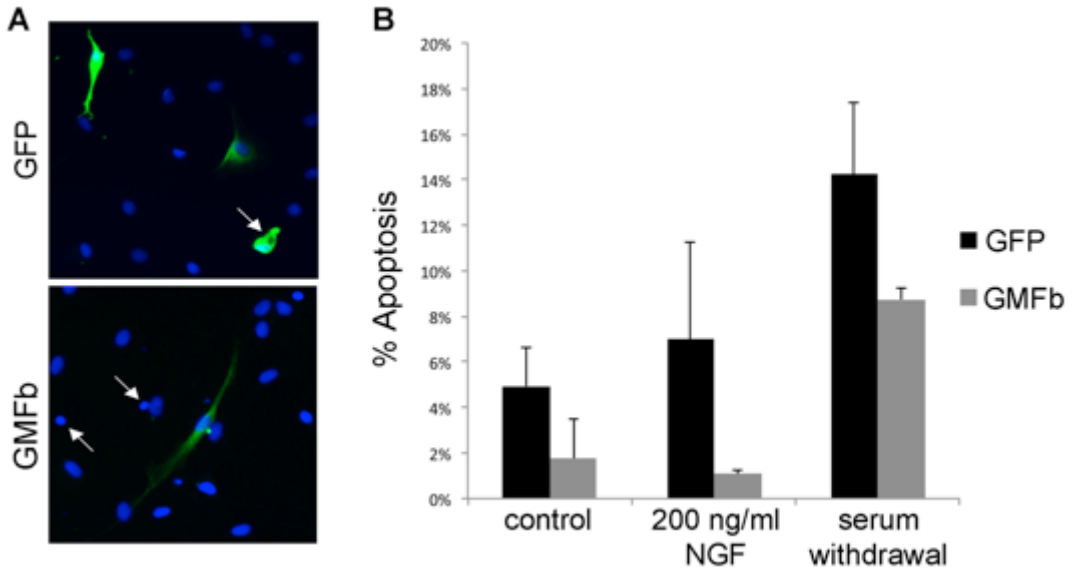


Figure 2: Rat Schwann cells were transfected with GMF-ß or GFP and then cultured in DMEM + 10% FBS. After 48 hours of incubation, media was changed to FBS-free DMEM (serum withdrawal) or high dose NGF was added (200 ng/ml). After 20 hours, cells were fixed and labeled with Dapi. The percentage of apoptotic cells (punctate Dapi) was counted.

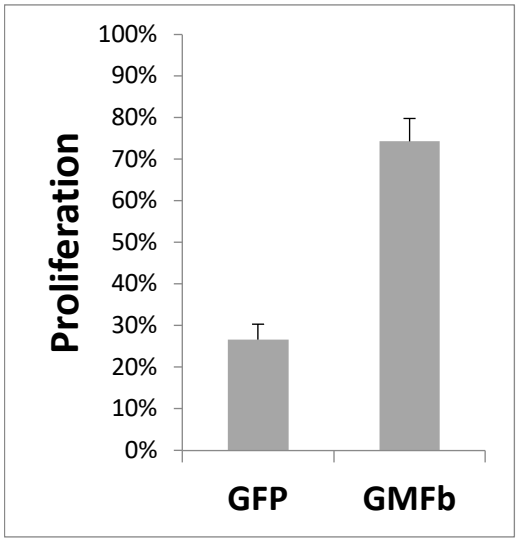


Figure 3: GMF-ß transfected Schwann cells proliferate faster than GFP-transfected Schwann cells.

Update since last quarterly progress report: We then developed a non-viral delivery method to deliver the GMF-ß plasmid into Schwann cells. We tested more than 10 different nanoparticle delivery formulations in vitro and in vivo to optimize the delivery and making sure the released plasmids were taken up by the Schwann cells and the GMF-ß was biologically active. Table 1 shows the properties of the different groups of nanoparticle-GMFß combinations.

Table 1		
Group #	**Nanoparticle + GMFß groups**	**Functionalization sequence**
1	FNC 1% PEG-g-lPEI/GMFb nanoparticles(N/P 8) –G1	Functionalized directly on nanoparticle
2	FNC 1% PEG-g-lPEI/GMFb nanoparticles(N/P 8) -G10	
3	FNC1% PEG-g-lPEI/GMFb nanoparticles (N/P 8) -G12	
4	FNC 1% PEG-g-lPEI/GMFb nanoparticles(N/P 8) -G14	
5	FNC 1% PEG-g-lPEI-G1/GMFb nanoparticles(N/P 8)	Functionalized polymer first
6	FNC 1% PEG-g-lPEI-G10/GMFb nanoparticles(N/P 8)	
7	FNC 1% PEG-g-lPEI-G12/GMFb nanoparticles(N/P 8)	
8	FNC 1% PEG-g-lPEI-G14/GMFb nanoparticles(N/P 8)	
9	FNC 1% PEG-g-lPEI/GMFb nanoparticles (N/P 8)	PEG-lPEI Control
10	FNC lPEI 22k/GMFb	lPEI Control
11	Bulk mixing: Polyplus in vivo JET PEI/GMFb nanoparticles	Polyplus positive control
12	Naked DNA (GMFb)	Negative control

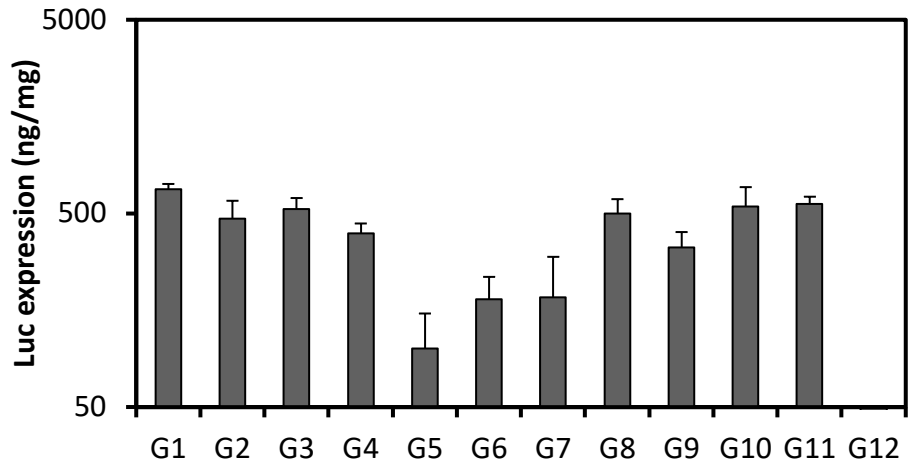


Figure 4: Transfection efficiency of various nanoparticle-GMFß formulations in vitro.

We then transfected tibial nerves with formulations from groups 1, 2, 7, 8, 11 and 12 (negative control). Both groups 1 and 8 gave similar results (Figure 5).

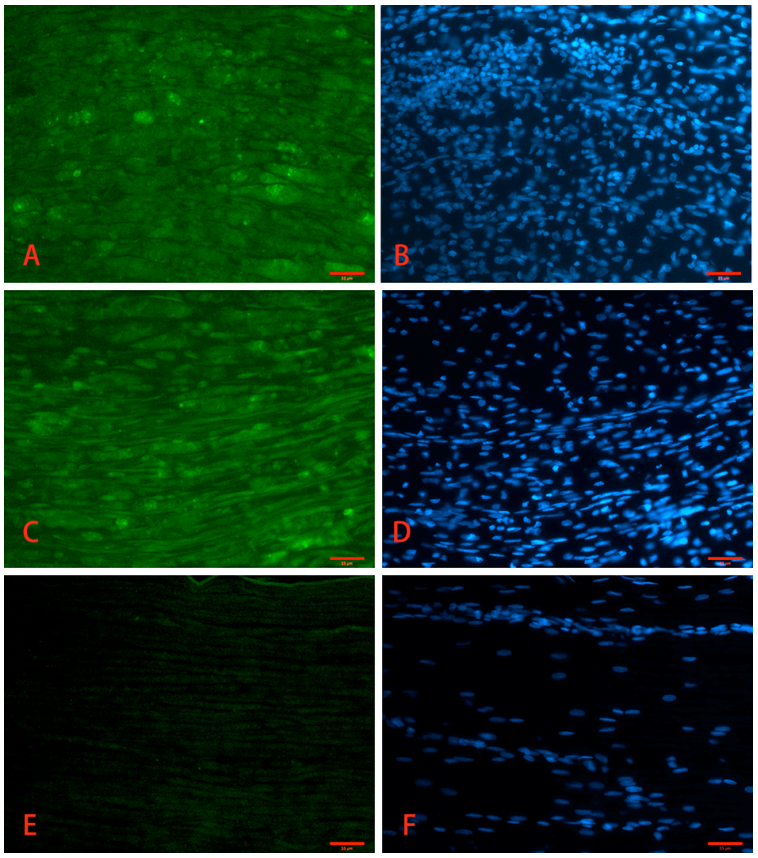


Figure 5: GMFß expression in tibial nerve 1 week after injection (A, C and E) and DAPI (B,D, and F). Both groups 1 (A) and 8 (B) showed good expression compared to plasmid alone group (E).

Task 1.4: To examine if the expected decline in Schwann cell numbers or markers of reactivity (e.g. levels of c-Jun, p75, erbB2 etc.) are prevented when GMF-ß is delivered to a denervated tibial nerve using nanoencapsulated particles in an established model of chronic denervation and regeneration

Milestone: nano-particle delieverd GMF-ß will prevent Schwann cell atrophy in vivo

Progress: We are currently carrying out the chronic denervation experiments to see if nanoparticle-GMFß combinations from Groups 1 and 8 are able to prevent atrophy and death of Schwann cells during chronic denervation.

Major Task 2: To maintain re-innervation capacity of chronically denervated muscles using sustained delivery of IGF-1

Task 2.1: To optimize the loading, stability and release kinetics of IGF-1 to achieve sustained release for 12 weeks

Milestone: Optimization of release kinetics in vitro

Progress: Given the difficulties in getting biologically active GMF-ß for Task 1, in order not to delay the project, early on during the project we tackled task 2.1. In order to have long term release of biologically active IGF-1 we used a new method for nano-encapsulation as shown in Figure 6:

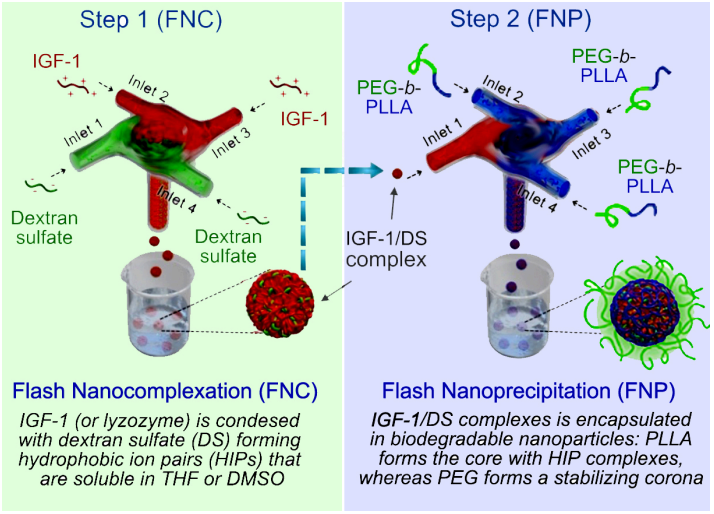


Figure 6: Nano-encapsulation method for proteins

Figure 7 shows characterization of the representative IGF-1 nanoparticles:

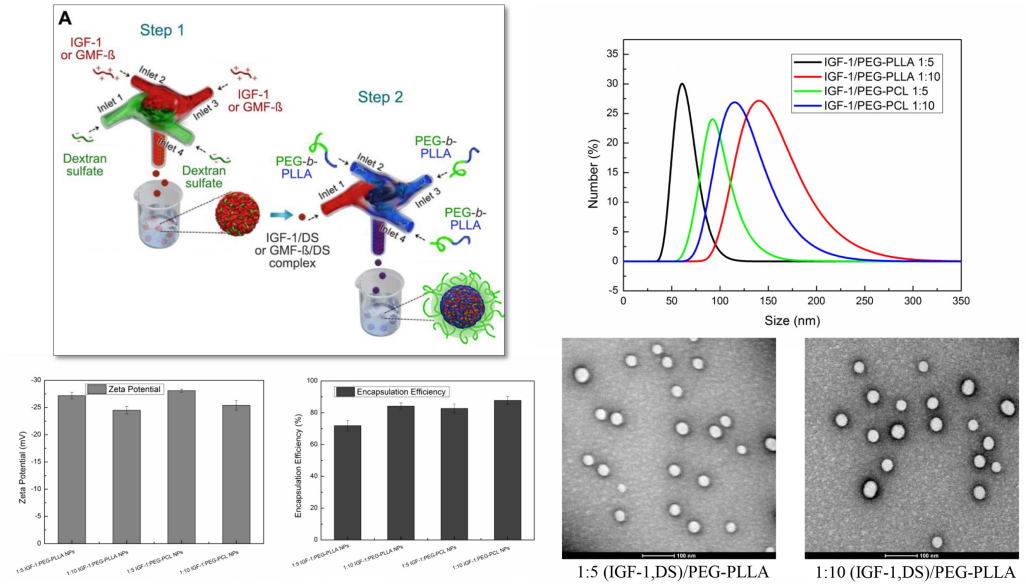


Figure 7: characterization of IGF-1 nanoparticles

As seen in Figure 8, different size (flow rate), protein/polymer mass ratio do not alternate the release rate significantly. So, we can vary the particle size and coating ratio to achieve high drug loading and local retention of NPs without affecting the release profile. Furthermore, we found that PEG-PCL matrix has slower release rate than PEG-PLLA.

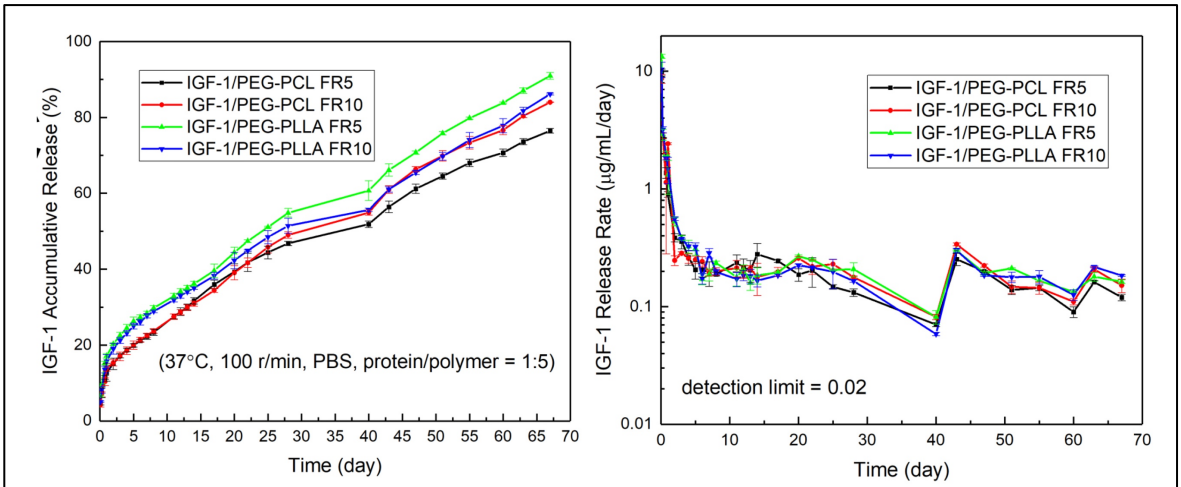


Figure 8: Effect of flow rate and polymer on release kinetics of IGF-1

Furthermore, we validated that the nano-encapsulation protocol did not affect the biological activity of the IGF-1 (Figure 9).

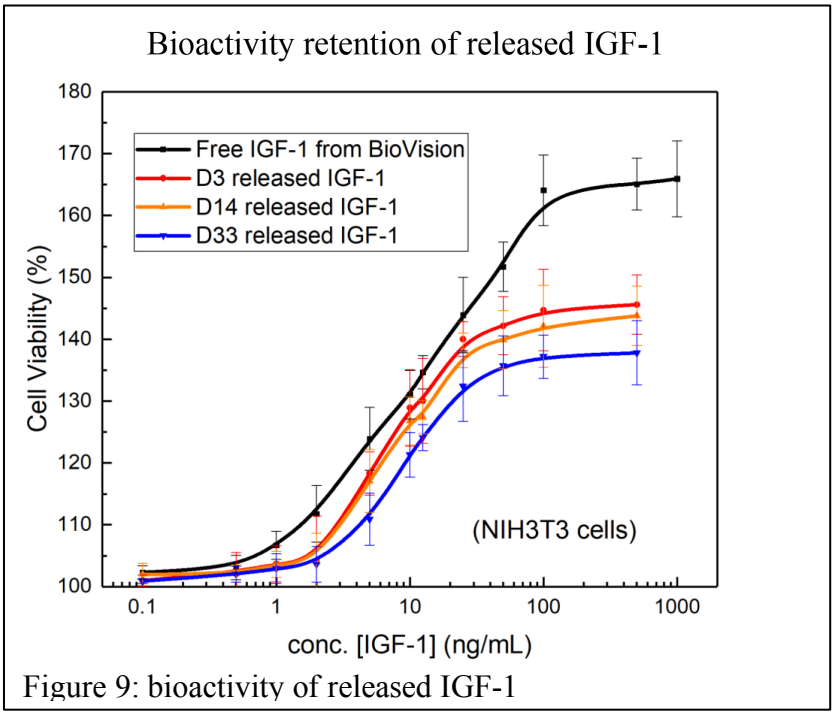


Figure 9: bioactivity of released IGF-1

Using these data, we calculated the dose to be injected in Task 2.2. Effective concentration (EC50) of IGF-1 is ~10 ng/mL. After a single injection of NPs containing 0.3 mg/mL IGF-1, the calculated decay of IGF-1 concentration is shown in Figure 10. A steady state concentration of 2 mg/mL is obtained. If the volume in the muscle is 2.5 mL, to reach a 60 days of release, we will need 1.5 ug of IGF-1. It asks for less than 0.02 mg of NPs for injection (consider the loading level to be ~10%, half of the theoretical value), which is achievable. Thus, the drug amount for a single injection can support an effective concentration for 2 months, given the release profile is nearly zero-order.

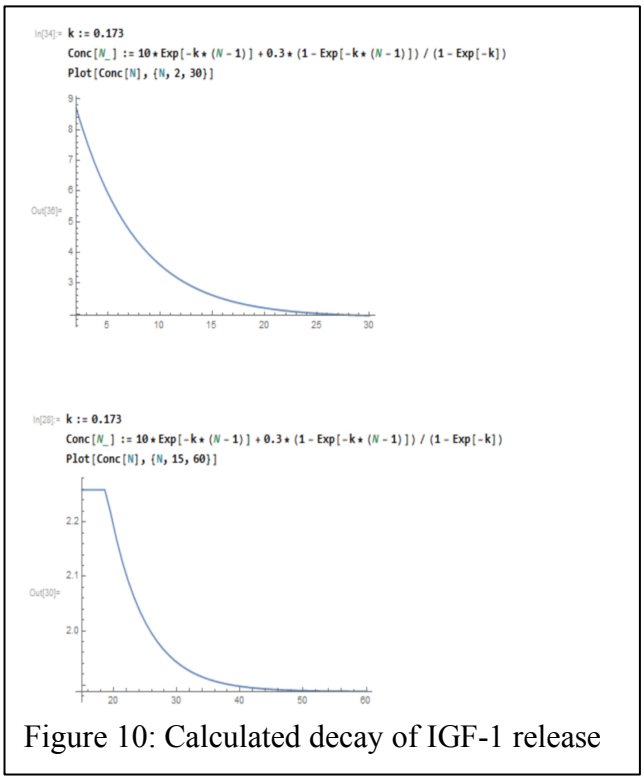


Figure 10: Calculated decay of IGF-1 release

Task 2.2: To examine changes that occur in the muscle during chronic denervation that is treated with slow release IGF-1 (we will evaluate muscle weight, myofiber size, neuromuscular junction (NMJ) integrity and gene expression changes that occur during denervation.

Number of animals: 30 rats (10 per group: albumin vs IGF-1 low dose vs IGF-1 high dose)

Milestone: nano-particle delivered IGF-1 will prevent muscle atrophy in vivo

Progress: These experiments were started during Q1 and were completed prior to Q3 report. As shown in Figure 11, wet muscle weight is better maintained in high dose IGF-1 group compared to low dose IGF-1 or control groups.

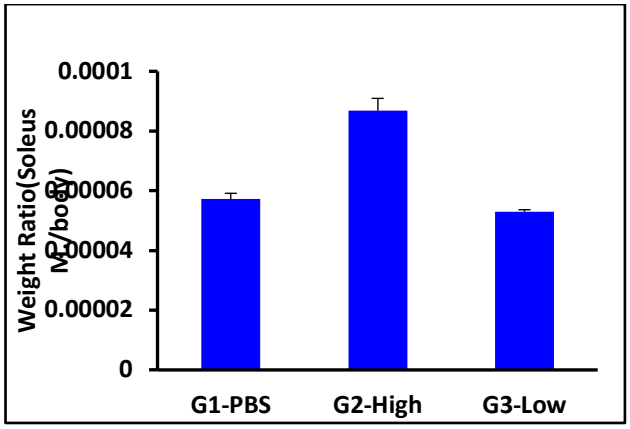


Figure 11: Ratio of soleus muscle to total body weight in mice treated with nano-encapsulated IGF-1 (high or low dose) into the soleus muscle 3 months after denervation.

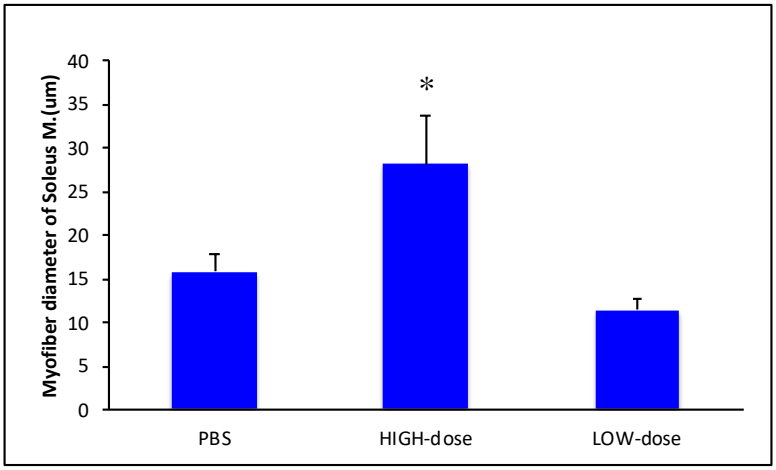


Figure 12: Myofiber diameter of soleus muscle after 3 months of chronic denervation.

Task 2.3: To correlate the release kinetics of IGF-1 and myofiber and NMJ maintenance during chronic denervation and optimize the IGF-1 nanoencapsulation process to achieve long lasting support to muscle during chronic denervation.

Number of animals: 30 rats (10 per group: albumin vs IGF-1 low dose vs IGF-1 high dose)

Milestone: Optimization of release kinetics in vivo and demonstration that maximum muscle atrophy is prevented

Progress: These experiments were started during Q1 and were recently completed. As seen in figures 11 and 12, administration of high dose of IGF-1 resulted in less muscle atrophy during

chronic denervation. After reinnervation surgery, the recovery of compound motor action potential was higher in the high dose IGF-1 group compared to other two groups (Figure 13). Furthermore, the high dose IGF-1 group had larger myofiber diameters indicating better reinnervation (Figure 14). Histological evaluation of NMJ reinnervation is ongoing and will be completed prior to next task. Nevertheless, these findings confirm that IGF-1 delivered to muscle has a beneficial effect in maintaining muscle size and improving reinnervation after repair surgery. Once Task 1 with GMF-in Schwann cells is completed we will use high dose IGF-1 for the combined approach in Task 3.

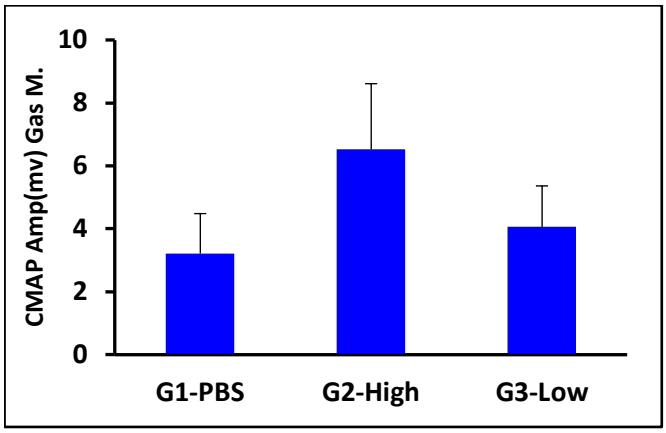


Figure 13: Amplitude of CMAPs (compound motor action potentials) recorded in the gastrocnemius muscle in IGF-1 treated mice. The tibial nerve was transected and the distal gastrocnemius muscle was left denervated for 3 months during which time it received nano-encapsulated IGF-1 (high or low dose) or control vehicle. After 3 months of denervation, the tibial nerve was repaired and regeneration was allowed for 2 months. The CMAP recordings were done after 2months and before tissues were harvested.

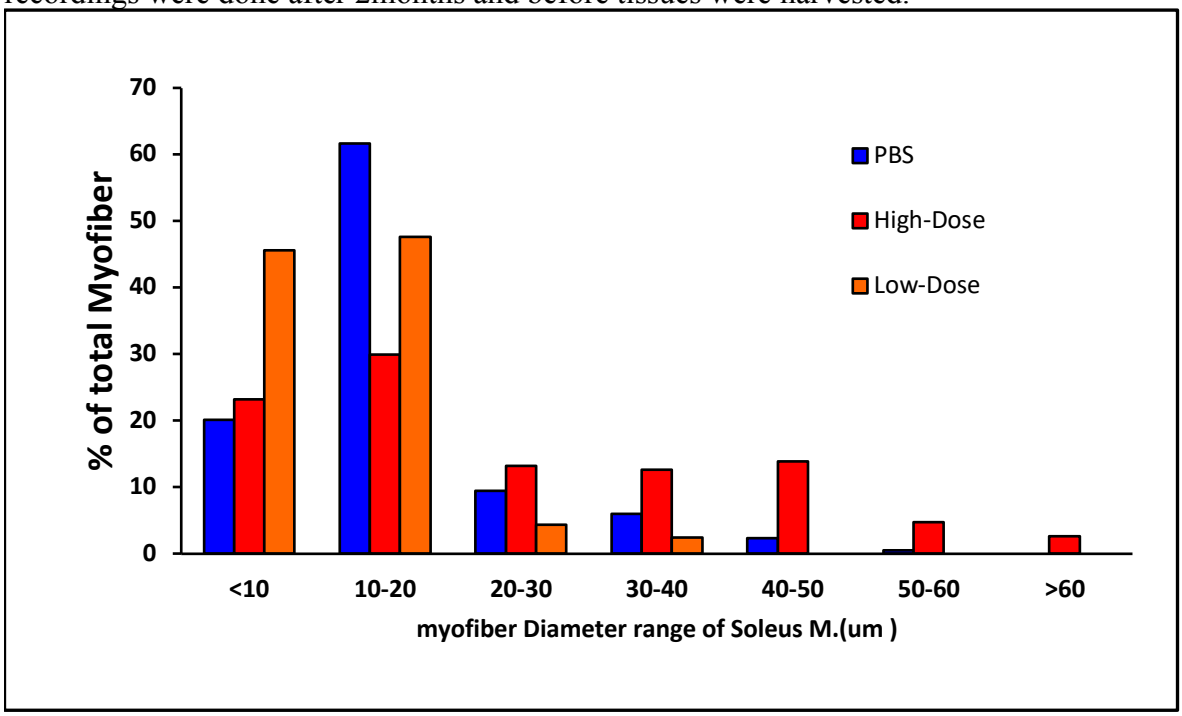


Figure 14: histogram of myofiber diameters indicate that high-dose IGF-1 had many larger diameter myofibers.

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

We plan to complete Task1.4 by Quarter Report #5 and then start Task 3

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).

We developed a novel method for combining biomolecules (IGF-1) and nanoparticles to release the biomolecule slowly over more than a month in a linear fashion while maintaining biological activity. This method can be further developed and validated with other biomolecules.

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.

As stated above, our new technique can be used in other disciplines where a slow release of biologically active large biomolecules is desirable.

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:

As communicated above and in prior quarterly progress reports, we had difficulties in Task 1 but we finally have a good plan and are in the process of completing this Task.

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.

Please see above explanation regarding Task 1. We had difficulties with commercial sources of GMFß and even when we made our own and demonsytrated biological activity in vitro, once it was encapsulated, the relased GNFß did not maintain biological activity. Hence we had to develop a new approach with delivery of GMFß gene to derive protein expression and maintain Schwann cells during denervation.

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.

Since multiple approaches were needed to accomplish Task 1, we had extra costs but those were covered by other funding sources in PI's lab.

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 (we have not disseminated our new technology yet)

- **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: *Ahmet Hoke*
Project Role: *Principal Investigator*
Researcher Identifier (e.g. ORCID ID): *0000-0003-1215-3373*
Nearest person month worked: *0.4*
Contribution to Project: *Supervised the project, wrote the IACUC and ACURO protocols.*

Name: *Hai-Quan Mao*
Project Role: *Co-investigator*
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: *0.4*
Contribution to Project: *Supervised the project*

Name: *Jami Scheib*
Project Role: *Postdoctoral Fellow*
Researcher Identifier (e.g. ORCID ID): *0000-0003-1215-3373*
Nearest person month worked: *6.0*
Contribution to Project: *Carried out the animal surgeries.*

Name: *Ruifa Mi*
Project Role: *Research Associate*
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: *3.0*
Contribution to Project: *Carried out the animal surgeries and histological analysis (replaced Dr. Scheib)*

Name: *Chenhu Qiu*
Project Role: *Postdoctoral Fellow*
Researcher Identifier (e.g. ORCID ID): 0000-0003-1215-3373
Nearest person month worked: *1.5*
Contribution to Project: *Carried out the drug formulation studies*

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.

Post-doctoral fellow, Dr. Jami Scheib has left Hopkins to start her independent career. In the last quarter, Dr. Ruifa Mi, who is an experienced peripheral nerve regeneration researcher took over her role in the project.

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*

Nothing to report

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to **https://ers.amedd.army.mil** for each unique award.

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

9. APPENDICES:

Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.

Quad chart is attached as Appendix

Strategies to Improve Chronic Nerve Regeneration and Target Reinnervation

OR160032

W81XWH-17-1-0698

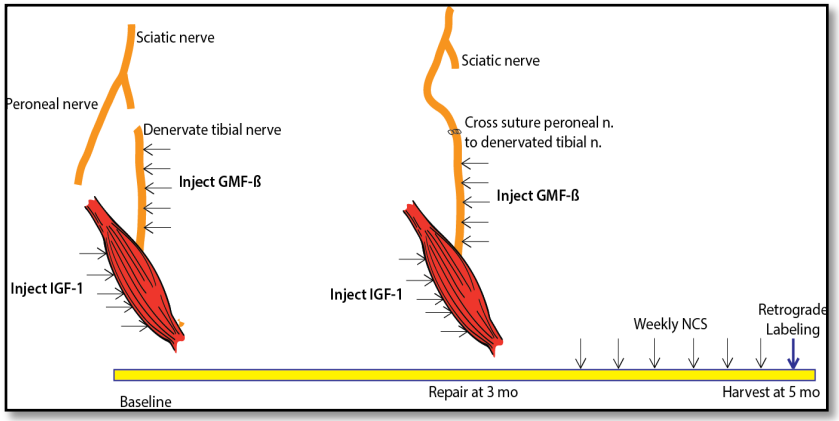
PI: Ahmet Hoke **Org:** Johns Hopkins University **Award Amount:** $499,978.00

Specific Aims

- Specific aim 1: To prevent atrophic changes in chronically denervated Schwann cells using GMF-ß and improve nerve regeneration
- Specific aim 2: To maintain re-innervation capacity of chronically denervated muscles using IGF-1 and improve functional recovery after peripheral nerve injury
- Specific aim 3: To improve functional recovery after chronic denervation using GMF-ß and IGF-1, delivered to the nerve and muscle, respectively

Approach

In this study we will use nano-encapsulated GMF-ß to prevent Schwann cell atrophy and IGF-1 to prevent muscle atrophy during chronic denervation, and achieve better functional outcomes after delayed nerve repair.



Accomplishment: Our preliminary data shows that GMF-ß is a critical factor that maintains Schwann cell survival and levels of IGF-1 are markedly reduced in chronically denervated muscle. We have also shown that we have the expertise to encapsulate growth factors in nanostructures as drug delivery platforms while maintaining biological activity

Timeline and Cost

Activities CY	17-18	18-19
To prevent atrophic changes in chronically denervated Schwann cells using GMF-ß		
To maintain re-innervation capacity of chronically denervated muscles using sustained delivery of IGF-1		
To improve functional recovery after chronic denervation by sustained local release of GMF-ß and IGF-1, delivered to the damaged nerve and target muscle, respectively		
Estimated Budget ($K)	**$250K**	**$250K**

Updated: (October 29, 2018)

Goals/Milestones

CY17 Goal – Validation of GMF-ß and IGF-1 efficacy independently

☐ Manufacture nano-encapsulated GMF-ß and IGF-1

☐ Implantation of GMF-ß into denervated nerve

☐ Implantation of IGF-1 into denervated muscle

CY18 Goal – Combined efficacy of GMF-ß and IGF-1

☐ Evaluation of combined implantation of GMF-ß and IGF-1

Comments/Challenges/Issues/Concerns

- We found that commercial sources of GMF-ß do not have adequate biological activity. We made our own and validated bio-activity but found that encapsulated GMF-ß has reduced bioactivity when slowly released. We then developed a new technique to deliver the GNFß plasmids for autocrine synthesis and support of denervated Schwann cells.

Budget Expenditure to Date

Projected Expenditure: $249,989

Actual Expenditure: $252,861