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TITLE: Understanding and Enhancing the Regenerative Capacity of Skeletal Muscle to Trauma by Targeting Muscle-Nerve Synergy

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CONTRACTING ORGANIZATION: University of Michigan, Ann Arbor, MI

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Fort Detrick, Maryland 21702-5012

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E-Mail:

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UNIVERSITY OF MICHIGAN
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13. SUPPLEMENTARY NOTES

14. ABSTRACT: Poor healing after lower-limb extremity trauma represents an enormous medical problem ($400B / year ascribed to trauma in CONUS and >24M limited duty days in 2005) and recent conflicts in Iraq and Afghanistan have emphasized the prevalence of lower-limb extremity trauma (up to 78 percent of medical disability discharges). These injuries present debilitating consequences, which have been shown to result in pronounced disabilities ranging from declines in limb function, to development of osteoarthritic pathology and delayed or elected limb amputation. Moreover, the effects of lower-limb extremity trauma have significantly reduced Department of Defense (DoD) readiness and performance and as each force begins to downsize, the importance of sustaining Warfighter readiness and recovery from trauma is a priority. Objective/ Hypothesis: While efforts to develop treatments that hasten and improve healing for lower-limb skeletal muscle injuries are ongoing, their development has been inherently limited due largely to our lack of understanding of the basic processes involved in the healing process. Efficient and appropriate repair and regeneration of skeletal muscle is mediated by a pool of muscle stem cells (MuSCs) called satellite cells, which activate, proliferate and differentiate and fuse to form new multinucleated myofibers. After regeneration of myofibers, function must be established by re-formation / attachment of a neuromuscular junction (NMJ). The NMJ connects the axon of a motor neuron to a muscle fiber and is responsible for excitation / contraction coupling and voluntary motor function. The intricate interaction between MuSCs and the NMJ niche is not fully understood and as such, how neural control influences response to trauma remains an open question.Specific Aims and Study Design: The overarching purposes of this project are to understand how the regenerative capacity of MuSCs are altered when the NMJ is disrupted and to utilize this niche to enhance MuSC-based therapies against traumatic injuries. Accordingly, we will perform three specific aims: Aim 1) Identify intrinsic factors in muscle stem cells that modulate muscle repair and regeneration after disruption to the neuromuscular junction. We have developed an animal model that permits study of NMJ disruption and MuSC response and will use this model to administer severe trauma followed by isolation of purified populations of MuSCs using flow cytometry.

15. SUBJECT TERMS

None listed.

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			USAMRDC
Unclassified	Unclassified	Unclassified	Unclassified	24	**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.*

The repair and regeneration of severely damaged soft tissues such as skeletal muscle remains a substantial clinical challenge and relatively few treatments exist. The overarching purposes of this project are to understand how the regenerative capacity of muscle stem cells are altered when the neuro-muscular junction is disrupted and to utilize this niche to enhance muscle stem cell-based therapies against traumatic injuries.

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

Neuromuscular Junction, Satellite Cells, Motor Neurons, Regeneration, Single Cells, Signaling

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: Establish mechanistic insights into the intrinsic molecular mechanisms and signaling pathways that couple skeletal muscle regeneration and neural control.

Major Task 2: Evaluate how manipulations to the NMJ can influence MuSC transplantation and functional regeneration following traumatic injury.

Major Task 3: Evaluate whether co-delivery of potent neurotrophic factor and MuSCs via an engineered biofunctional hydrogel synergistically augments regenerative capacity and functional recovery following traumatic injury.

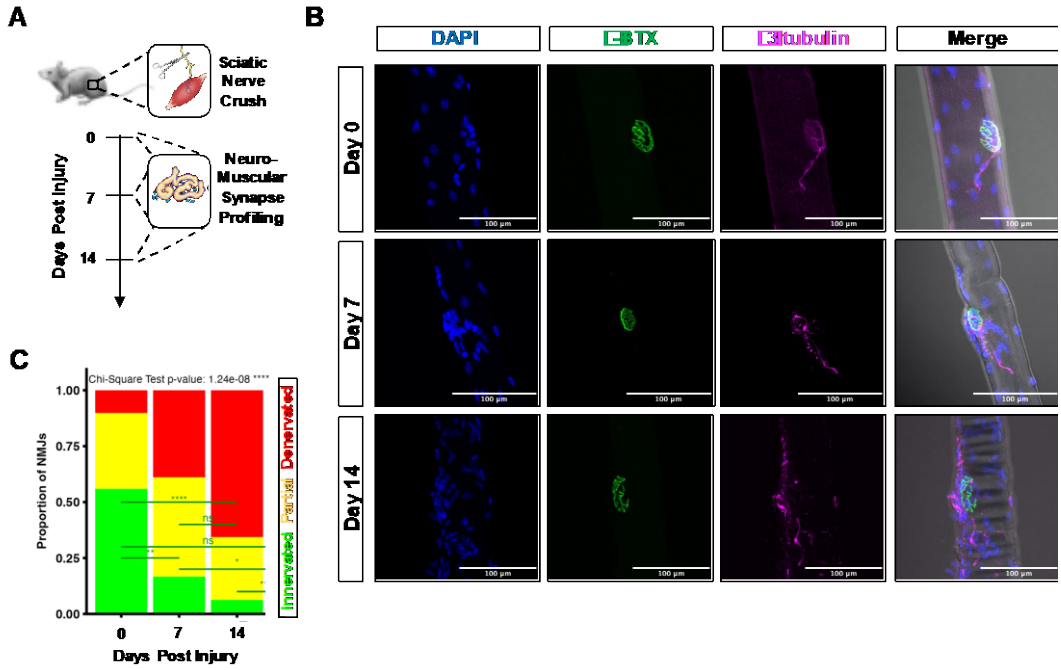
What was accomplished under these goals?
For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of 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 Task 1 – Key Experiments
Quantitative morphological characterization of mouse denervation and re-innervation kinetics after nerve injury.

- Quantified pre- and post-synaptic overlap as a metric of denervation after nerve injury using confocal microscopy and immunohistochemistry
- Developed and optimized single-nucle RNA sequencing assay to understand how sub-synaptic myonuclei, other myonuclei and other cell types change their gene expression at the single nucleus level at two key time points (7 days and 14 days) post injury
- Performed bioinformatics analysis of single-nucle RNA sequencing datasets and evaluated how denervation sensitive population changed

Summary of major findings
- Sciatic nerve crush injury results in acute denervation followed by reinnervation
- Single nuclei sequencing revealed muscle stem cells and sub-synaptic myonuclei exhibit similar patterns of expression during re-innervation suggesting a role for MuSCs to support NMJs by donating mitochondria to reduce oxidative stress.

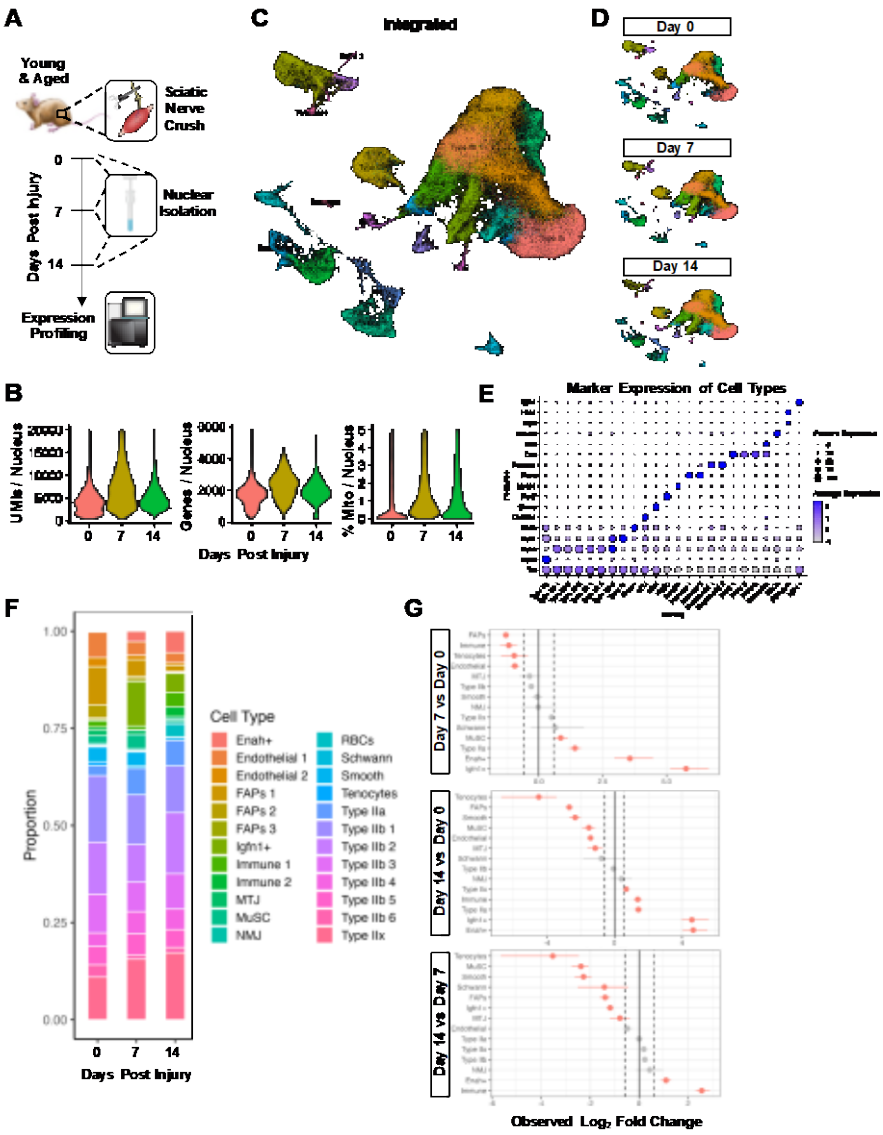
Major Task 1 – Subtasks 2-3: Crush injury of sciatic nerve results in skeletal muscle denervation.
We have established that administration of a reproducible model of nerve injury by surgical exposure of the sciatic nerve and mechanical pinching using forceps for 10 seconds resulted in denervation followed by re-innervation (**Fig. 1A**). To understand the kinetics of this process, we extracted tibialis anterior (TA) muscles before (0 days), and 7 and 14 days after nerve injury. We triturated single myofibers, fixed and immunostained the cells and used confocal immunofluorescence (IF) microscopy and 3-D image rendering to assess the presence of nerve terminal markers (β-3 tubulin), and post-synaptic receptors (acetylcholine receptor-AChR, **Fig. 1B**). The NMJs were quantitated as innervated if >80% of post-synaptic regions (AChRs) are covered by pre-synaptic markers (β-3 tubulin), and denervated if the postsynaptic apparatus had negligible if any labeling of pre-synaptic terminal markers (**Fig. 1C**). We observed progressive increases in fully denervated and partially denervated myofibers for days 7 and 14 when compared to uninjured myofibers (**Fig. 1C**). These results confirm our injury model results in denervation and how myofibers respond to recruit motor neurons back.



Major Task 1 – Subtasks 2-3: Figure 1. Quantitative assessment of re-innervation after nerve crush injuryu injury. **A)** Experimental schematic for administration of sciatic nerve crush injury followed by morphological profiling of the neuro-muscular junction over a time course. **B)** Representative confocal images of the neuromuscular synapse before and after sciatic nerve crush injury immunostained for pre- and post-synaptic compartments. Blue-DAPI, Green-alpha Bungarotoxin, Pink – Beta 3 Tubulin. Scale = 100µm. **C)** Quantitation of images from B) showing overlap of pre-and post-synaptic compartments.

Major Task 1 – Subtasks 2-3: Profiling changes in expression of single nuclei after nerve injury reveals alterations in myonuclei

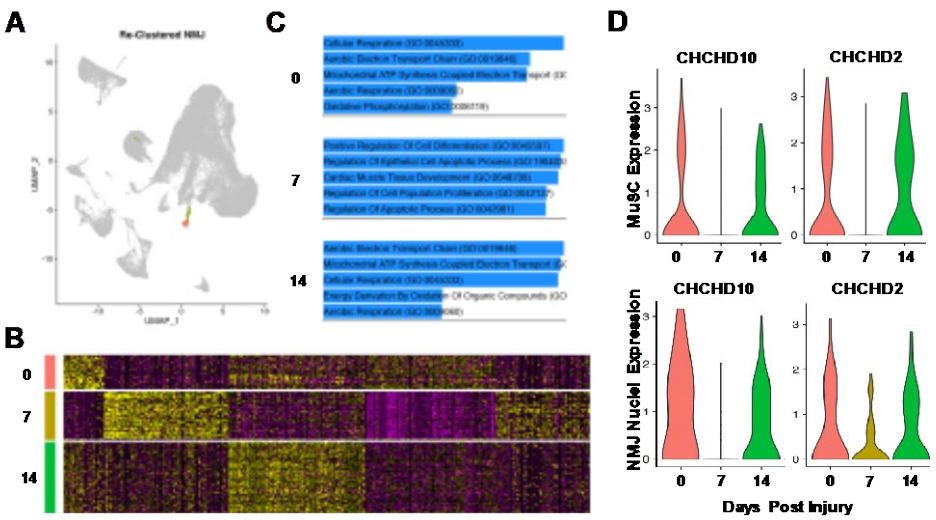
To probe the molecular underpinnings of changes in the post-synaptic response to nervous injury, we performed droplet-based RNA-Sequencing on single nuclei (snRNA-Seq) isolated from uninjured (0 days post injury or dpi) as well as 7 and 14 dpi from young (3 months) tibialis anterior muscles (**Fig. 2A**). We generated 75,114 total high-quality snRNA-Seq libraries encompassing on average 1,980 genes and 4,765 unique molecular identifiers (UMIs) per nucleus (**Fig. 2B**). Ambient RNA was removed using SoupX and technical variations between batches were regressed out using Seurat followed by dimensionality reduction using Uniform-Manifold Approximation and Projection (UMAP). Unsupervised Louvain clustering revealed 16 cell types (**Figs. 2C-D**), which were annotated by matching unique marker genes with previously published datasets (**Fig. 2E**). The datasets revealed variation in the number and type of captured nuclei in denervated muscles when compared to uninjured, whereby new myonuclear populations were significantly increased in denervated muscles at days 7 and 14 (Enah+ and Igfn1+ as well as Type IIa myonuclei) when compared to uninjured myonuclei (**Figs. 2F-G**). Igfn1+ and Enah+ myonuclei have previously been observed after injury or during development and suggest significant intracellular communication to promote re-innervation.



Major Task 1 – Subtasks 2-3: Figure 2. Single-nuclei expression profiling before & denervation show differential responses of muscle stem cells & post-synaptic myonuclei. **A)** Schematic of experiment design, whereby hind limb muscles from young (3 mo) mice were injured via sciatic nerve crush. Extraction of in-tact nuclei and sequencing before (day 0), and after 7- and 14-days injury. n=4 biologically independent animals / age / time point. **B)** Quality control metrics for captured single nuclei. **C)** Integrated unsupervised clustering plot by uniform manifold approximation (UMAP) embedding of cell types collected from different ages and time points colored by cell type. **D)** UMAP plots displayed from each time point. **E)** Dot plot of cell-type-specific marker gene expression where dot size represents # cells per group expressing the gene listed and the color indicates level of expression. **F)** Quantification of cell abundances at each time point. **G)** Fractions of cell types recovered compared for abundance for each time point.

Major Task 1 – Subtasks 5-6: Sub-synaptic myonuclei change expression of mitochondrial genes after nerve injury to promote re-innervation

Our single-nuclei gene expression profiles revealed capture of myonuclei that have previously been described to be sub-synaptic (expressing AChRs such as Chrne). We re-clustered the NMJ myonuclei (**Fig. 3A**) and used PRESCIENT, a bioinformatics tool to evaluate time-series RNA-sequencing datasets from single cells, to quantitatively understand differentially expressed genes through time (**Fig. 3B**). Pathway annotation of differentially expressed genes at 7 days revealed proliferative and differentiation genes. At 14 days post injury, significant alterations in metabolism and mitochondria were detected when compared to uninjured muscles. Focusing in on mitochondrial genes, we detected that both MuSCs and sub-synaptic myonuclei increased expression of coiled-coil-helix-coiled-oil-helix- domain 10 (CHCHD10, **Fig. 3D**), which is an inner-membrane mitochondrial protein that is mutated in neuropathies such as ALS, Charcot–Marie–Tooth and late-onset spinal motor neuropathy. CHCHD10 is essential for ATP production and specifically enriched at the NMJ, and mutation of CHCHD10 has been found to result in mitochondrial fragmentation. We found that CHCHD10 and CHCHD2, the homolog protein of CHCHD10, were both differentially expressed and given these factors are critical regulators of oxidative phosphorylation, these results suggest a role for MuSCs to support NMJs by donating mitochondria to reduce oxidative stress.



Major Task 1 – Subtask 5: Figure 3. Muscle denervation induces a fibrotic response from fibro-adipogenic progenitors that impinges on muscle stem cells. A) Highlight of NMJ myonuclei overlaid on integrated UMAP. **B)** Heatmap of differentially expressed genes for NMJ myonuclei for each time point. **C)** Enriched GO or Reactome pathways for each time point. **D)** Gene expression violin plot of CHCHD10 and CHCHD2, post-synaptic mitochondrial proteins, in muscle stem cells (top) and post-synaptic myonuclei (bottom) showing dynamic expression during re-innervation.

Major Task 2 – Key Experiments

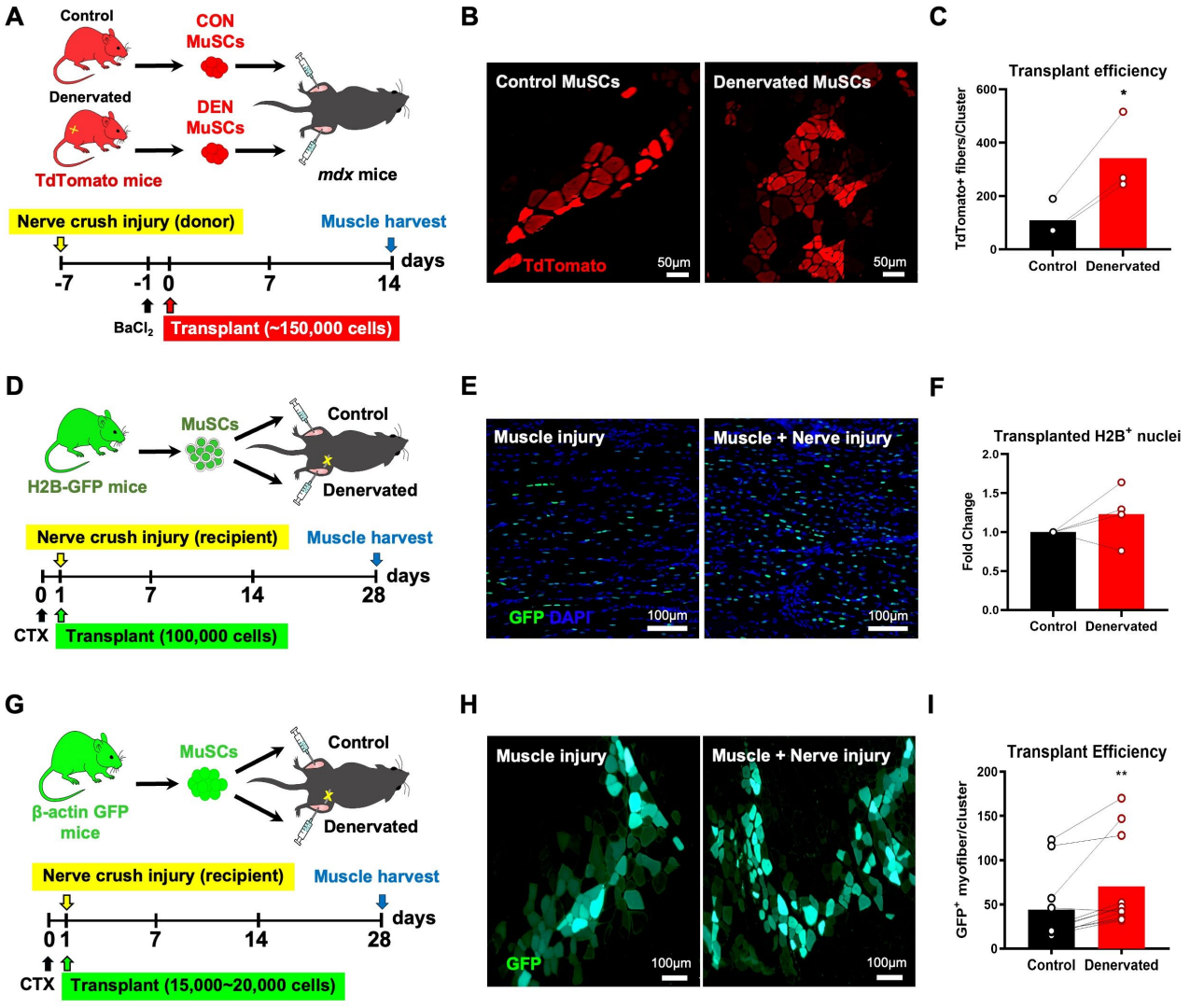
Evaluate how manipulations to the NMJ can influence MuSC transplantation and functional regeneration following traumatic injury.

- Assess whether denervation injury (NMJ manipulation) alters muscle stem cell function and regeneration.
- Assess engraftment of donor and recipient effects of MuSCs following NMJ perturbation.

Summary of major findings

- Muscle stem cells isolated following 7 days of NMJ perturbation exhibit significantly enhanced transplant efficiency.
- Single nuclei sequencing revealed muscle stem cells and sub-synaptic myonuclei exhibit similar patterns of expression during re-innervation suggesting a role for MuSCs to support NMJs by donating mitochondria to reduce oxidative stress.

Major Task 2 – Subtasks 1-2



Nerve perturbation promotes a favorable microenvironment that augments muscle regeneration

We postulated that the positive enrichments in muscle stem cell (MuSC) function induced by nerve injury would result in concomitant enhancements in muscle regeneration. Thus, we tested whether the denervation affected the muscle tissue environment of the recipients to improve MuSC transplant efficiency. To verify enhanced muscle regeneration in the nerve-injured muscle, we transplanted an equal number of GFP^+ donor MuSCs into nerve plus muscle injury or muscle injury only recipients (G, H, and I) After 28 days of transplantation, TA muscles were harvested and analyzed for engraftment efficiency. As expected, denervated host muscle displayed a significantly higher number of GFP^+ myofibers, suggesting that nerve injury synergistically augmented myogenesis of exogenous MuSCs. Conversely, when an equal number of control and 7 day-denervated MuSCs ($TdTomato^+$) were transplanted into the barium chloride injured-recipient muscles, the donor MuSCs from denervated muscle showed significantly greater engraftment compared to that of control muscle *ex vivo* (A, B, and C). To further confirm our hypothesis that the increased muscle regeneration is due to newly added myonuclei and subsynaptic nuclei from the MuSCs, we transplanted the same number of MuSCs, isolated from H2B-GFP reporter mice, and traced the number of $H2B^+$ myonuclei in control and denervated muscles but they were not statistically significant to each other (D,E, and F). These results demonstrate that changes in the neuromuscular niche promote regenerative dynamics of MuSCs in muscle fibers.

Major Task 3 – Key Experiments

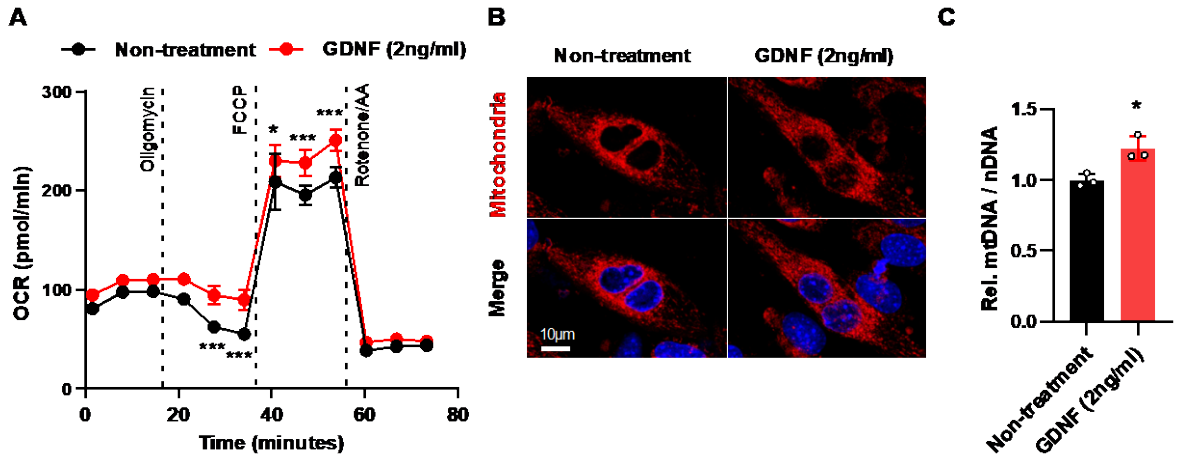
Evaluate whether co-delivery of potent neurotrophic factor and MuSCs via an engineered biofunctional hydrogel synergistically augments regenerative capacity and functional recovery following traumatic injury.

- The role of glial-derived neurotrophic factor (GDNF) on myogenesis

Summary of major findings

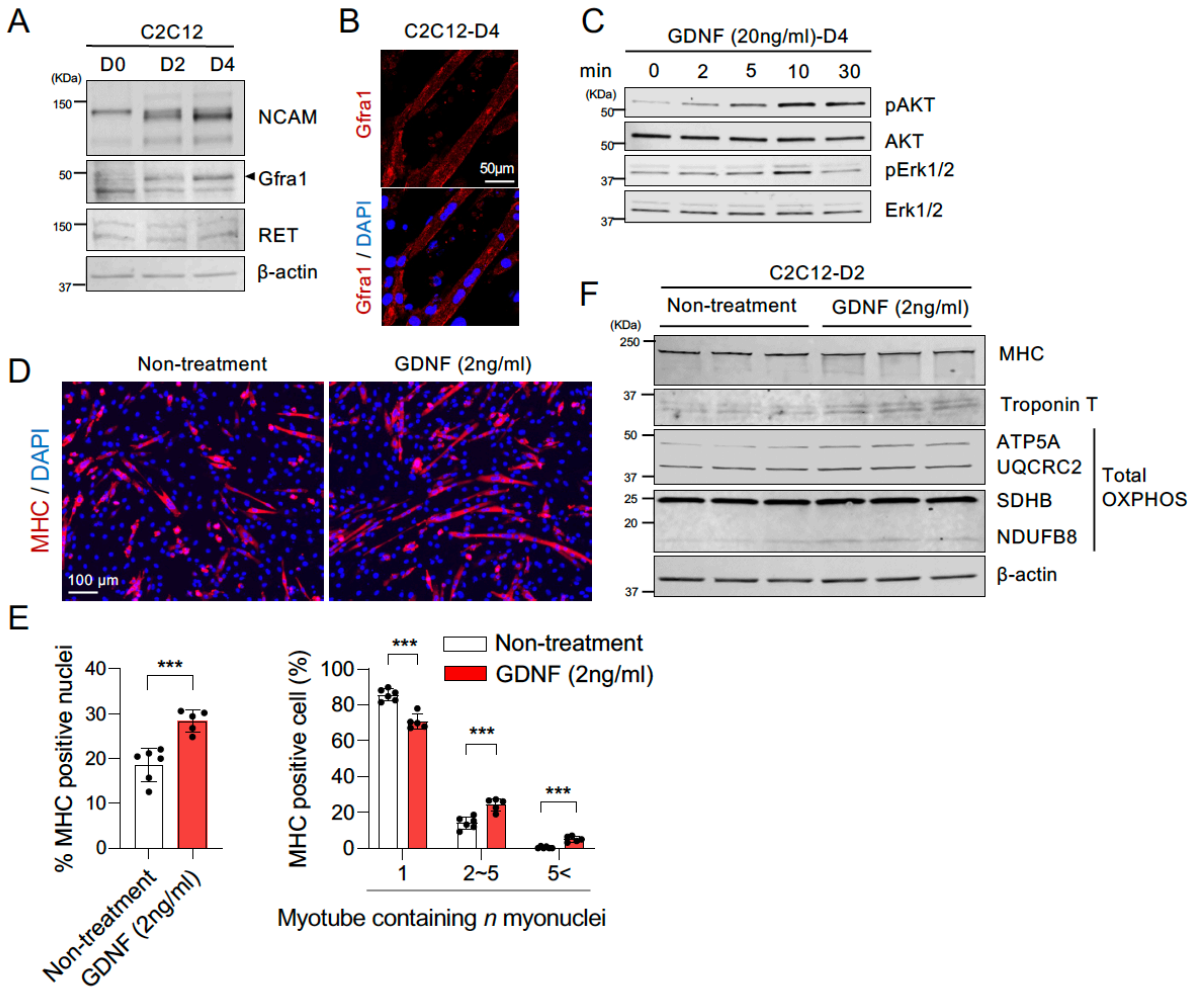
- Exposure of MuSCs to recombinant GDNF correlates with enhanced mitochondrial bioavailability & anabolic signaling
- Muscle stem cells deliver mitochondria to damaged neuromuscular junctions.

Major Task 3 – Subtask 1: Glial-derived neurotrophic factor enhances myogenesis through mitochondrial biogenesis. We previously established that glial derived neurotrophic factor augmented MuSC differentiation and fusion. Our single-nuclei sequencing experiments suggested MuSCs and sub-synaptic myonuclei participate in re-innervation through donation and changes in mitochondrial signaling. To further understand this mechanism, we treated MuSC derived myoblasts with GDNF recombinant protein and measured mitochondrial content as well as function using the Seahorse assay (**Fig. 5A**). The basal respiration level of MuSCs revealed no differences between myoblasts with and without GDNF treatment, but ATP-linked respiration through treatment with oligomycin and the maximal respiration (FCCP treated) showed significant elevations in GDNF treated myoblasts (**Fig. 5A**). Furthermore, recombinant GDNF treatment significantly increased mitochondrial oxygen consumption rate (**Fig. 5A**). GDNF treatment also resulted in increased mitochondrial content compared to non-treated MuSCs (**Fig. 5B-C**). These results suggest that MuSCs may respond to neural perturbations by local release of GDNF and adjusting their regenerative behavior to support the NMJ.



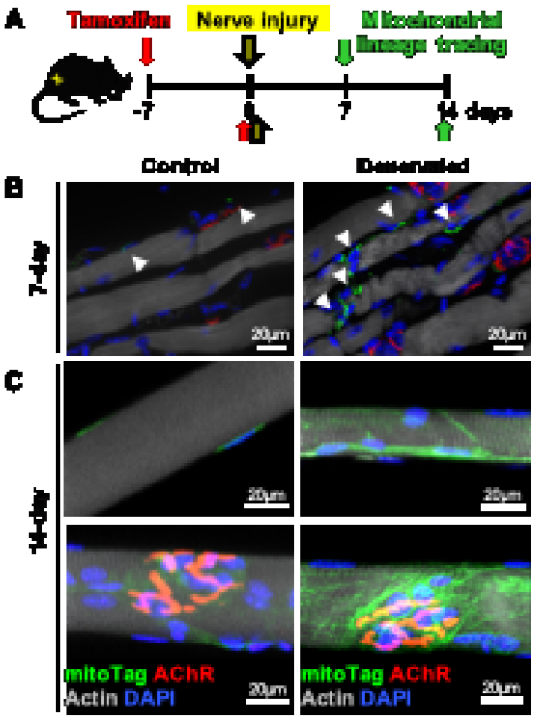
Major Task 3 – Subtask 1: Figure 5. Myoblasts respond to recombinant GDNF through mitchondrial. A) Seahorse mitochondrial stress test. No differences were seen in basal respiration, but ATP-linked respiration (through oligomycin treatment) and maximal respiration (FCCP treated) show significant elevations in GDNF treated myoblasts. Furthermore, recombinant GDNF treatment significantly increased mitochondrial oxygen consumption rate. **B)** Immunostaining of mitochondrial DNA content in primary myoblasts. Scale = 10um. **(C)** Quantitation of images from B) showing enhanced mitochondrial content relative to nuclear DNA for GDNF treated myoblasts. *p<0.05 & n=3 biological replicates.

Major Task 3 – Subtask 1: Glial-derived neurotrophic factor enhances myogenesis through mitochondrial biogenesis. To further determine how GDNF influenced MuSCs and myogenesis we used C2C12s, an immortalized myogenic cell line, and quantified which neurotrophic receptors are present on myogenic progenitors. We found that GDNF receptor components, NCAM and GFra1, are upregulated during myogenic differentiation (**Fig. 6A-B)**. Given these two receptors promote the anabolic signaling pathways AKT and ERK, we performed Western blots to determine the effect of recombinant GDNF treatment on C2C12 myoblasts (**Fig. 6C)**. Since changes in AKT and ERK have been shown to increase myogenic differentiation, we differentiated the myoblasts into myotubes, immunostained and imaged myosin heavy chain (MHC+) for GDNF and untreated samples (**Fig. 6D**). Consistent with our previous observations, we observed GDNF promoted an increase in MHC+ myotubes and myotubes with more nuclei (**Fig. 6E-F**). We further validated that GDNF treatment increased mitochondrial electron transport chain (oxidative phosphorylation) components by Western blot substantiating that neural signaling through local changes in GDNF promotes a positive microenvironment for MuSCs to enact regeneration.



Major Task 3 – Subtask 1: Figure 6. Myoblasts respond to glial derived neurotrophic factor by increasing anabolic signaling and oxidative phosphorylation resulting in increased differentiation and fusion. To determine how a neurotrophic factor influenced MuSCs and myogenesis we focused on the neurotrophic factor, glial-derived neurotrophic factor (GDNF). We used C2C12s, an immortalized myogenic cell line, and quantified which neutrophic receptors are present on myogenic progenitors. We found that **(A)** and **(B)** GDNF receptor components, NCAM and GFra1, are upregulated during myogenic differentiation. **(C)** Anabolic signaling pathways AKT and ERK are activated by recombinant GDNF treatment in C2C12 myoblast. **(D)** GDNF treatment increases myogenic differentiation as measure by myosin heavy chain (MHC)+ myotubes. **(E)** Right, Quantification of total number of MHC myotubes. (Left) Fusion index assessed by number of nuclei in MHC+ myotubes. (F) GDNF treatment increases mitochondrial electron transport chain (oxidative phosphorylation) contents as measured by Western blot.

Major Task 3 – Subtask 1: Muscle stem cells deliver mitochondria to the neuromuscular junction in response to nerve injury. Given the influence of GDNF on mitochondria in myogenic progenitors, we reasoned one of the mechanisms MuSCs may promote functional recovery is delivery of healthy mitochondria to the NMJ. We administered a peripheral nerve injury and found increases muscle stem cell derived mitochondrial content proximal to the NMJ (**Fig. 7A**). We used a MuSC mitochondrial lineage tracing model whereby MuSC mitochondria contain a fluorescent reporter (eGFP) in the outer mitochondrial membrane (Pax7 Cre^{ERT}-MitoGFP). The mice were fed with tamoxifen chow for 7 days before neve injury to activate Cre-based recombination and expression of GFP in MuSC mitochondria. Nerve injury significantly increase mitochondrial content around neuromuscular junctions at 7 and 14 days post injury (**Fig. 7B-C**). Representative images of interfibrillar mitochondria and subsynaptic mitochondria confirm our hypothesis that link muscle regeneration to neural control and suggest a coupling of healthy MuSCs to NMJ homeostasis.



Major Task 3 – Subtask 1: Figure 7. Muscle stem cells deliver mitochondria to damaged neuromuscular junctions. A) Experimental schematic of system to trace mitochondria from MuSCs (Pax7^{CreERT}-Rosa26$^{CAG\text{-}LSL\text{-}EGFP\text{-}OMM}$ or Pax7-MitoTAG). Pax7-MitoTAG mice harbor an enhanced green fluorescent protein (eGFP) in the outer mitochondrial membrane and were given nerve injury via sciatic nerve injury & imaged at 7 and 14 days post injury. **B)-C)** Representative images of single myofibers at 7 and 14 days after injury where MuSC-derived mitochondria/GFP are detected proximal to NMJ regions. NMJs (BTX, AChRs-Red), DAPI (blue), GFP (green). Scale = 20um.

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.

We previously published results from Major Task 1 in eLife, which is an open access journal. These results were presented at the Myogenesis Gordon Research Conference by Paula Fraczek, the graduate student working on Major Task 1.

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.

Major Task 1: Establish mechanistic insights into the intrinsic molecular mechanisms and signaling pathways that couple skeletal muscle regeneration and neural control.

In the next year, we will complete bioinformatics analysis of myonuclei after denervation and try to connect how changes in their intracellular signaling converge onto muscle stem cells that help promote re-innervation and muscle regeneration.

Major Task 2: Evaluate how manipulations to the NMJ can influence MuSC transplantation and functional regeneration following traumatic injury.

In the upcoming year, to assess whether repeated nerve stimulation activate myogenic activities of muscle stem to enhance regeneration, we will utilize optogenetic stimulation (ChR2 transgenic mice) to mimic nerve injury and examine, muscle stem cell activation, proliferation, and differentiation *in vitro* and *in vivo*.

- Optimize optogenetic stimulation of motor neurons using blue light (488 nm) that enhances myogenic activity of muscle stem cells.
- Quantify $Pax7^{+}$ MuSC, MuSC activation, proliferation, and differentiation.
- Assess muscle regeneration with or without optogenetic stimulation.

Major Task 3: Evaluate whether co-delivery of potent neurotrophic factor and MuSCs via an engineered biofunctional hydrogel synergistically augments regenerative capacity and functional recovery following traumatic injury.

In the next year, to understand synergistic effects neurotrophic factors and muscle stem cell transplantation. We will test the following:

- Evaluate the optimal dose of GDNF delivered within biofunctional hydrogel that maximizes muscle regeneration.
- Assess whether co-delivery of GDNF and MuSCs enhance muscle regeneration following sciatic nerve injury
- Determine whether multiple co-delivery of GDNF and MuSCs (every 2 months for 4 times) attenuate age-associated deficits in NMJ degeneration and improve contractile function.

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).

Nothing to report

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

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

Nothing to report

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

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

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

Nothing to report

What was the impact on society beyond science and technology?

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:*

Dr. Jang moved universities from Georgia Tech to Emory in 2021-2022 and this has delayed progress on Major Tasks 2 and 3. He has initiated a new animal protocol and ACURO approval and this has taken time to perform.

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.

None

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.

None

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

None

Significant changes in use of biohazards and/or select agents

None

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).*

  1) Larouche, J. et al. Muscle Stem Cell Response to Perturbations of the Neuromuscular Junction Are Attenuated With Age. *eLife* **10**, e66749 (2021).
  2) Choi, JJ et al. Motor neuron injury primes muscle stem cells for myogenesis by enhancing protein synthesis and mitochondrial bioenergetics. *In preparation for submission* (2022)

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).*

None

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.*

None

- **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.

https://www.nobel.bme.umich.edu/

https://www.janglabemory.org/

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

None

- **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.

None

- **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.*

None

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: *Paula Fraczek*
Project Role: *Graduate Student*
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: *12*
Contribution to Project: *Ms. Fraczek has administered nerve trauma on transgenic animals, imaged single myofibers to glean muscle stem cell contributions and worked on isolating myonuclei from muscle for profiling.*

Name: *Jeongmoon Choi*
Project Role: *Graduate Student/Postdoc*
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: *12*
Contribution to Project: *Dr. Choi performed in vitro and in vivo experiments on nerve injured muscle stem cells, muscle stem cell transplantation, and prepared manuscript on these results.*

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.

No

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:
<u>Organization Name:</u>
<u>Location of Organization: (if foreign location list country)</u>
<u>Partner's contribution to the project</u> (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.*

Young C. Jang – Georgia Institute of Technology/Emory School of Medicine

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.