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14. ABSTRACT

In the proposed studies, we have used our experience in soft and hard tissue regeneration induced by the HIF1α-stabilizing drug, 1,4-DPCA, in a drug delivery system (PEG-DPCA nanogel) towards therapies for hand, nerve, and digit regeneration. In the current studies, we are exploring the effect of this drug on 1) digit regrowth post-amputation and nerve regeneration, 2) drug effects on peripheral re-innervation in rat forelimbs after injury and 3) optimization of drug potency and delivery in these systems. Our progress during this third year includes studies on surgically-amputated digits in mice using Micro-CT analysis showing changes with 1,4-DPCA drug therapy with and without BMP2. Significant changes at 3 months showed changes in bone density and volume. In the Tuffaha/Brandacher model of rat forelimb nerve resection, using larger rat cohorts than previously reported, we again showed drug-enhanced grip strength, reduced muscle atrophy, and nerve fiber regeneration.

During this no-cost extension year, we will explore multiple drug injections for longer periods of time, new dosing, effects of BMP2 addition post-digit amputation, continue studies in forelimb nerve regrowth, effects on digit function, further development of the 1,4-DPCA drug delivery system and modification of 1,4-DPCA compounds.

15. SUBJECT TERMS

BMP2; Bone Mineral Density; Bone Volume; 1,4-DPCA; Digit Amputation: Flexor digitorum profundus (FDP), Grip Strength Analysis; Micro-CT Analysis; Muscle Atrophy; Nerve Co-aptation; Nerve Regeneration

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1. Introduction

The studies presented here address a biomedical issue of importance to warfighters and the general patient population. We are using our experience in soft and hard tissue regeneration induced by a small molecule HIF-1a stabilizing-drug therapy (1,2) towards treatments of hand nerve injuries and digit regeneration. In this proposal, we are invoking classical regenerative responses rarely seen in mammals but which are common in lower species such as newts and salamanders that can readily regrow lost limbs. This proposal is focusing on the development of novel therapies to repair neurosensory damage, maintain the distal and organ interface, or regenerate the neuromuscular junction for re-innervation of end organs during peripheral nerve regeneration using a nerve resection model in rats (3,4) and a digit amputation model in mice in an attempt to restore macro-anatomic and functional digit and hand restoration through a classical regenerative process.

2. Keywords

BMP2; Bone Mineral Density; Bone Volume; 1,4-DPCA; Digit Amputation: Flexor digitorum profundus (FDP), Grip Strength Analysis; Micro-CT Analysis; Muscle Atrophy; Nerve Co-aptation; Nerve Regeneration

3. Accomplishments

What were the major goals of the project?
There are 4 major goals of this project as stated in the SOW:

1. We will determine the effect of drug/gel using different doses and added drug injections over time or any new drug formulations on nerve regeneration at terminal sites in the mouse digit after amputation.
2. We will examine healing across nerves ends after transection in the forelimb, and track nerve recovery in the rat using different injury models and drug dosages.
3. We will synthesize and characterize 1,4-DPCA-PEG Conjugates in vivo and in vitro.
4. We will synthesize and characterize 1,4-DPCA-Peptoid Conjugates.

What was accomplished under these goals?

Aim 1. Nerve regeneration and hard and soft tissue growth at sites in the proximal digit post amputation.

Experiment #4. We initiated a fourth group of studies (Exp #1-3 were presented in previous years) to determine if we could enhance the response we were getting. Given previous studies by Muneoka (5,6) and others, we decided to add BMP2 in a gelatin bead to the amputation site in addition to the DPCA injected systemically (day 0 and day 8) and subcutaneously. Again, the second phalanx of the middle digit of the hind-paw was surgically amputated midway between the proximal and distal joints under isoflurane and long-term buprenorphine which last for 3 days (see **Fig 1**). We used 2 mice/group and the groups consisted of 1) mice not given drug (control); 2) mice given drug subcutaneously; 3) mice given BMP2 locally on day 8 post-amputation; 4) mice given BMP2 locally on day 14 post-amputation; 5) mice given both DPCA + BMP2 Day 8; and 7) mice given DPCA + BMP2 Day 14. These mice were followed for 3 months using microCT analysis.

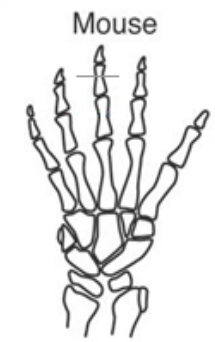


Figure 1

Micro-CT imaging of mice was performed using a Quantum GX Micro-CT imaging system (PerkinElmer, Hopkinton, MA, USA) and bone volume and mineral bone density determined. Volumes of digits between joint and amputation site were calculated using the Analyze 14.0 software (Analyze Direct, Overland Park, KS, USA). The bone mineral density was estimated using a hydroxyapatite phantom (QRM-MicroCT-HA, QRM GmbH, Möhrendorf, Germany).

Unfortunately, we lost 5 animals in this experiment early in the process. We believe that the combination of extended use of anesthesia (isoflurane) for the CT scanning + long-term buprenorphine + the amputation + the injection of drug done at the same time was too much for the mice. We have decided to do the scanning and the DPCA injection on day -1 in the future. However, we were able to generate some data from this experiment. As seen in **Fig 2**, the value at day 1 varied between mice because the measurements involved the distance between the joint and the amputation site (which varied between animals).

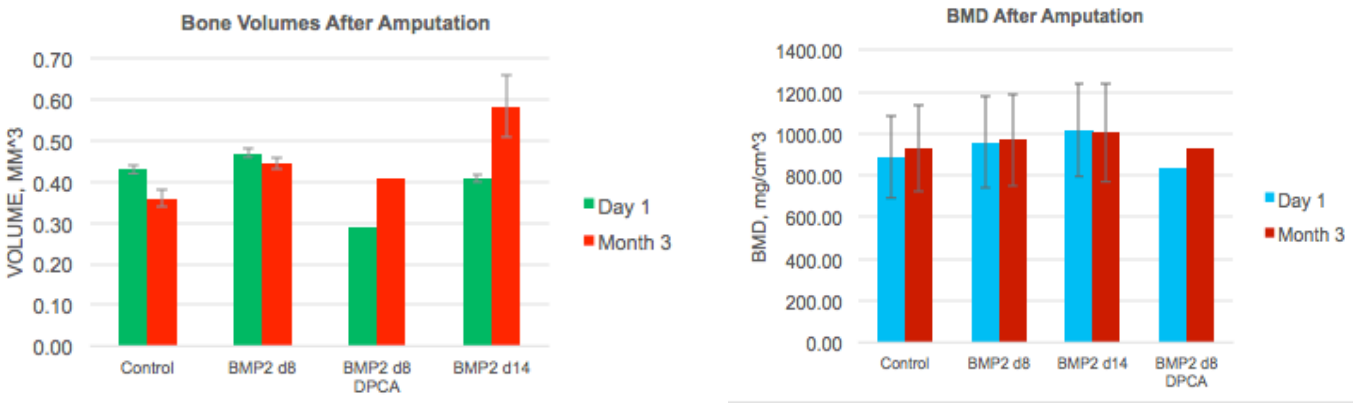


Figure 2. Bone changes were determined after digit amputation by measuring bone volume and bone mineral density at day 1 compared to 3 months. On the left figure, bone volume data from 2 mice for each group is presented with st. error bars. The controls show a reduction in bone volume over time (p=0.08) with no change after giving BMP2 alone on day 8 (p=0.3). An increase in volume was seen if the mouse was given DPCA then BMP2 on day 8 (1 mouse). A larger increase was seen in the two mice given BMP2 on day 14 (p=0.1). On the right side, bone density is seen. An increase is seen after BMP2 da8 + DPCA, however, as mentioned above, there was only 1 mouse. Future experiments will use more mice per group.

From micro-CT readings, on the left side of **Fig 2** is the bone volume and in the two control mice (first two bars), the volume is going down at 3 months. In the next two bars, mice were given BMP2 on day 8 post-amputation and there is little effect. The next set of bars show only 1 mouse which was given both DPCA and then BMP da 8. Here there is a 25% increase in volume, suggesting that DPCA is having an effect. The DPCA controls unfortunately died. On the other hand, BMP2 given later than day 8 (on day 14) without DPCA showed a 30% increase in volume. Obviously, we want to know if the two, DPCA and BMP2 work together. This experiment clearly needs to be repeated with more animals.

Aim 2. Healing across co-apted nerve ends after transection and tracking nerve recovery in Sprague Dawley rats.

In these experiments, rat nerves that control digit movement were severed, and nerve recovery examined. In this case, rats were examined every 10-20 days up to day 81. Grip strength, nerve regeneration, and muscle degeneration and regeneration were examined. We will in the future determine the presence and quantity of neuromuscular junctions.

Our goal was to determine the effect of drug treatment on nerve regeneration after nerve co-aptation.

The controls for the cutting of two nerves, the median and ulnar nerves, was to suture the nerves to the muscle so that no reconnection or regrowth is possible. This went through an evolution until no regain of function was found. Originally, the median and ulnar nerves were cut and left in place. Our pilot study showed that this surgical technique was inadequate, as rat peripheral nerves have the ability spontaneously heal across small gaps. We transitioned to cutting the nerves and suturing the proximal ends of the two nerves to nearby muscles, leaving the distal ends free. This transposition technique provided the intended results of no nerve regeneration (with rare exception; in three animals the sutured nerve came away from the muscle and was able to heal – we confirmed this during tissue harvest). Therefore, we most recently transitioned to suturing all 4 ends (two proximal and two distal) of the two cut nerves to nearby muscles to prevent any chance of unintentional reconnection, which has been successful in all cases of our most recent “no-repair” group.

The median and ulnar nerves were cut and repaired (co-apted) via an ulnar to median nerve transfer. In this technique, validated and repeatedly reported by the Tuffaha lab, the proximal ulnar nerve is coapted to the distal median nerve and that reliably allows for return of grip strength function. This is done because primary repair of these nerves, once cut, is under some tension and we wanted to avoid any risk that tension would impair healing (as is known to occur in nerve repairs). This technique was used in all of our "repair" animals, some receiving DPCA and others not ("controls").

Our primary measure of nerve recovery was grip strength. We used the limb stimulation approach developed in the Tuffaha lab to avoid known limitations with induced grip techniques. Under general anesthesia, SD rats were positioned with the arm in supine position. Two needle electrodes were inserted under the skin of the arm and placed on top of the nerve. The Chatillon Digital Force Gauge was attached to a trapeze shaped grasping bar. The grasping bar was placed in the palm of the rat. Stimulation via the electrodes prompted the rats to grip the grasping bar. The force gauge was then slowly pulled away from the rat and the highest measurement reading is recorded. The average of three trials was recorded. Baseline measurements were taken 1-3 days pre-operatively and every 7 days post-operatively until Day 81.

I. Grip Strength
II. Nerve survival and regeneration
III. Muscle Atrophy

I. <u>Grip Strength</u>

Four parameters were examined based on grip strength:

1. No DPCA treatment: Sutured to muscle (no repair) (Group 1) (Figure 3A)
2. DPCA treatment: Sutured to muscle (Group 3) (Figure 3B)
3. No DPCA treatment: Co-apted nerve (Group 2)(Figure 3C)
4. DPCA treatment: Co-apted nerve (Group 4) (Figure 3D)

1. In the Control Groups 1 and 3, nerve was sutured to the muscle, so that there was no nerve co-aptation and thus no grip strength recovery. Since two nerves were cut, both distally and at the carpal tunnel location, one or both of these were necessary for grip strength. As seen in **Fig 3A**, Group 1, nerve sutured to muscle, no recovery is seen up to day 81. In Group 3 (**Fig 3B**), again nerve was sutured to muscle. The rats were injected SQ with 1,4-DPCA/PEG at time of injury and again on day 8. Again, no regain of grip strength was seen.

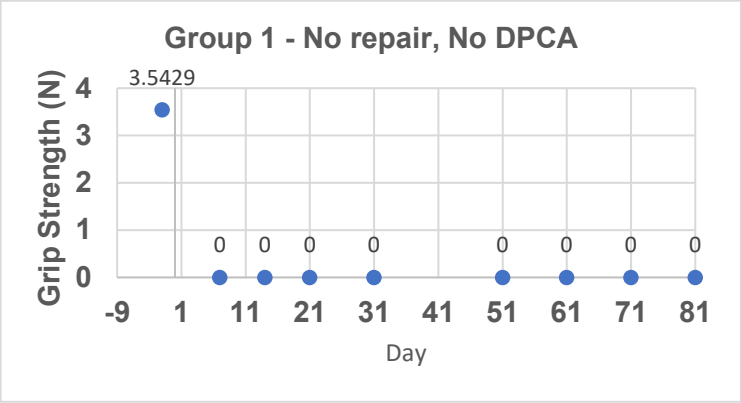


Figure 3A: Grip strength data of <u>No DPCA</u> treatment group #1: Nerves were sutured to the muscle so there is no possibility for regain in grip strength.

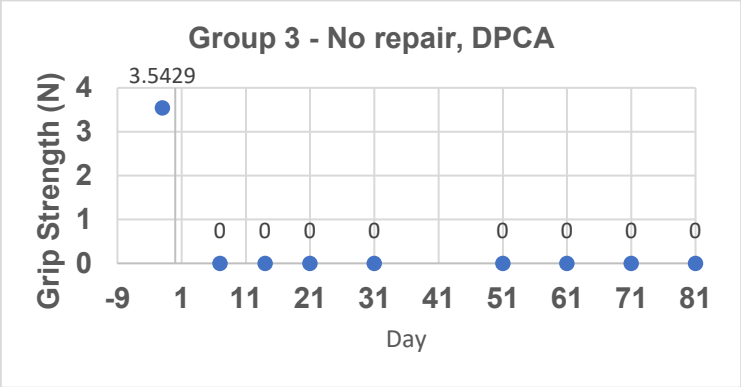


Figure 3B: Grip strength data of DPCA treatment group #3: Nerves were sutured to the muscle so there is no possibility for regain in grip strength.

2. In the co-apted Groups 2 (**Fig 3C**) and 4 (**Fig 3D**), either treated with DPCA or not, all animals regained grip strength beginning at Day 31 and continued to increase until day 81. As seen in **Fig 3E**, DPCA appears to be having an effect on days 61-81, similar to what we had seen in our preliminary experiment at later timepoints. This is consistent with degradation of distal nerve without drug (upper **Fig 4**).

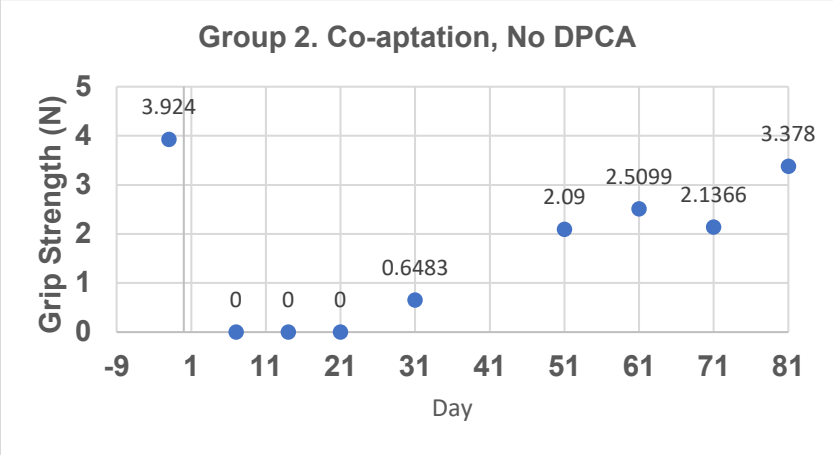


Figure 3C: Grip strength data of co-apted nerve Group #2. No DPCA is given. Regain of grip strength is seen between days 31 to 81.

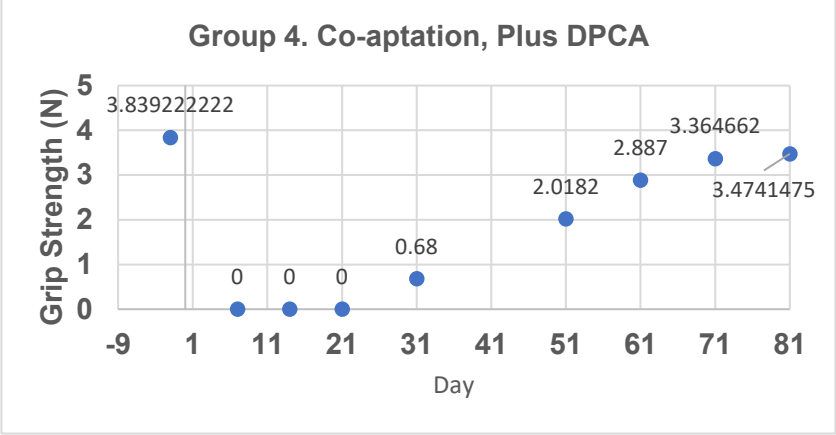


Figure. 3D: Grip strength data of co-apted group #4: DPCA SQ da 0 and da 8. Regain of grip strength was seen at days 31 to 81.

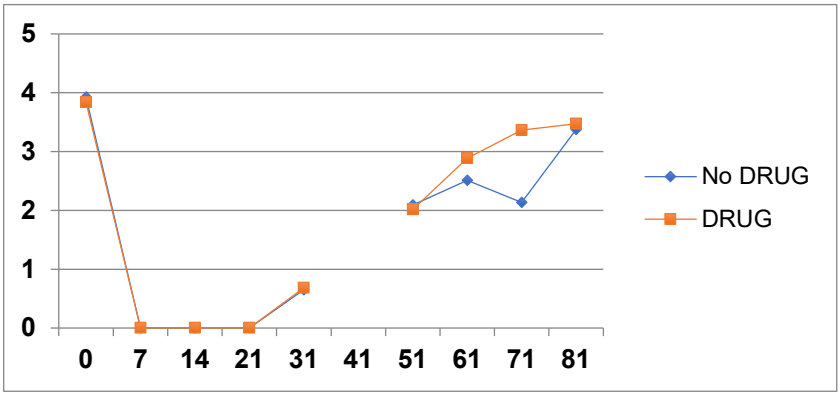


Fig 3E: Grip strength data from co-apted nerve injury groups comparing the effect of drug and no-drug on regain of function. The Y-axis is grip strength and the X-axis is days post-injury.

To increase the effect of DPCA, we will give drug not only immediately after injury but test whether an additional dose will have an effect 1 month or two months later.

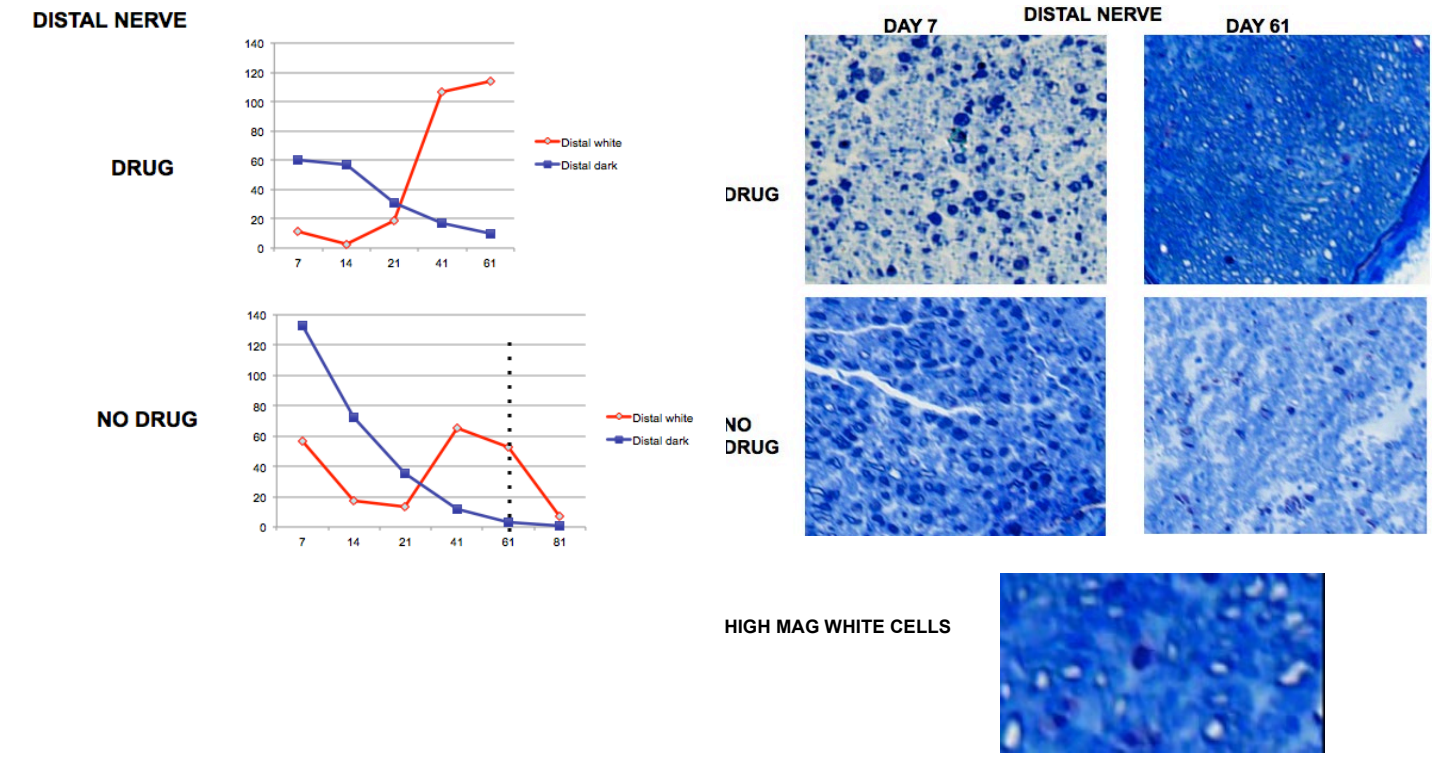
II. Nerve survival and regeneration

Biweekly samples were fixed, embedded, and cut 7-81 days after surgery, tissue sections from the surgical site were recovered. Slides were made and stained or unstained for further treatment and sent to Philadelphia for the Heber-Katz laboratory to carry out analysis of tissue and histochemistry and immunohistochemistry for nerve growth, muscle degeneration, neuromuscular junction analysis and scar formation.

During this grant period, slides from days 7, 14, 21, 41, 61, 81 were stained with toluidine blue (a myelin stain) and then counted in a given area (41 mM^2) for dead and dying nerve fibers due to Wallerian degeneration (brown) and for live and newly regenerating nerve fibers (white in center).

In the upper part of **Fig.4**, tissue and nerve fiber counts from co-apted nerve experiments are seen as graphs showing that there is a dramatic difference between early and late timepoints in the drug vs no drug group for the distal nerve. Distal nerve is the nerve section 1 cm distal to the coaptation site. At the early timepoints, without drug, the number of dying cells and live cells is higher compared to the Drug group where the number of dying cells and live cells is lower. We actually saw the same drug effect in the mouse ear pinna experiments along with increased proteases (MMPs) and inflammatory cells and ascribe this to the ability of drug to remodel tissue early and allow new structures to grow (ie. tear down the old bldg. to make room for the new one). At the later timepoints, we see a progressive increase in new live cells with drug. Without drug however, the tissue makes an effort to regrow new nerves but is less robust.

We also evaluated histology of the nerve in the carpal tunnel, many mm further distal from the "distal to coaptation" nerve specimen. In the lower part of **Fig 4**, examining the carpal tunnel nerve, a similar picture is seen, though the effects are not as severe. Thus, the early timepoints show some reduced though not dramatic level of both brown and white cells. And at the later timepoints, the curves are similar though with drug, the number of white cells are 30% greater.



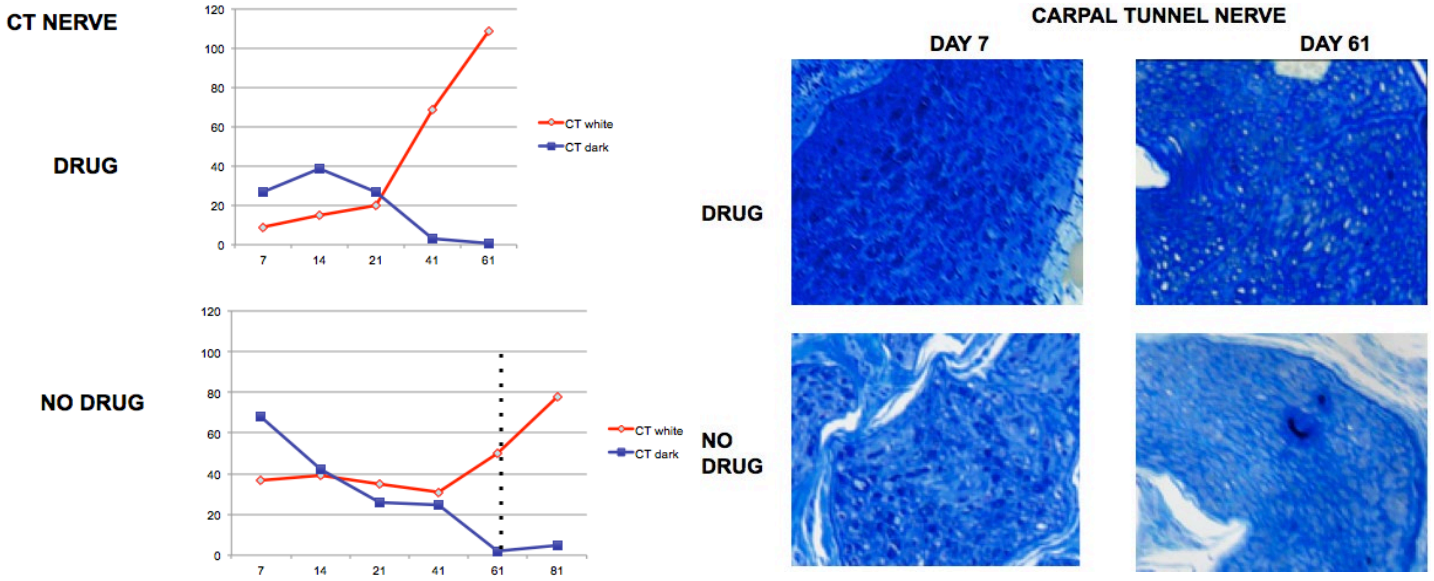


Figure 4. Co-APTED groups with DPCA (Group 4) vs No DPCA (Group 2). On the left are the graphs of the number of nerve fibers/41 mM^2 from nerve sections stained with a myelin stain. On the right, sections from day 7 compared to day 61 can be seen (day 81 no drug groups not shown). The blue lines represent brown cells which are dead or dying. The red lines represent live and newly generated white cells (the bottom-most picture shows white cells from day 61 plus drug under higher magnification).

The upper **Fig 4** data is derived from distal nerve tissue; the lower **Fig 4** data is derived from carpal tunnel nerve tissue. The Y-axis is the number of nerve fibers and the X-axis is the number of days post injury. The number of slides (n=2) and the number of sections per slide (n=2-4) were analyzed.

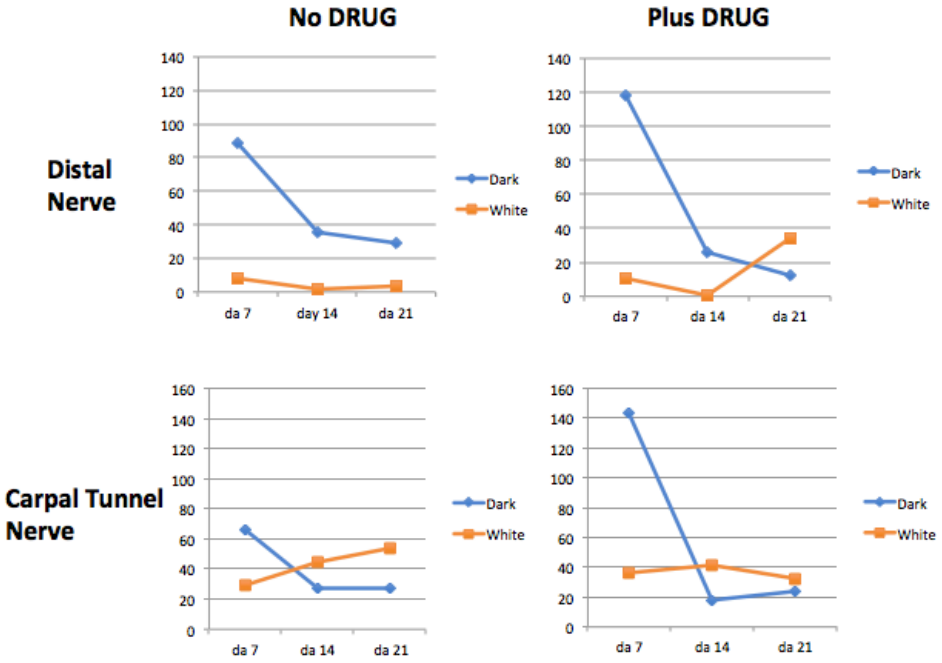


Figure 5. Sutured to Muscle: DPCA vs No DPCA. The graphs of dying cells (blue lines) vs live cells (orange lines) are seen for both distal and carpal tunnel nerve tissue with and without drug. Note, the timepoints here include days 7, 14, and 21. The Y-axis is the number of nerve fibers, and the X-axis is the number of days post injury. The number of slides (n=2) and the number of sections per slide (n=2-4) were analyzed.

In **Fig 5**, in nerve tissue from non-co-apted tissue (sown to the muscle) and from graphed counts of dying and live nerve fibers, the effect of drug remodeling early during the response is not seen. However, at later timepoints and with drug for the distal nerve, the number of white cells goes up even though the two sides of the nerve cannot grow together. Thus, the drug does have an effect on nerve cell regeneration starting on day 21 even though the tissue cannot reconnect.

In both surgical models of nerve transection, there is an obvious effect of drug on nerve regeneration. However, this is translated into a small change in recovery for grip strength. We hope to enhance this by giving more drug at later timepoints.

III. Muscle Atrophy

To examine the effect of injury on muscle and muscle atrophy, we stained muscle longitudinal sections with anti-laminin antibody and counted the muscle fiber diameter. As seen below, the groups with co-apted nerve show differences between rats treated with or without drug starting on wk. 6. Those animals given drug showed increased muscle fiber width, increased nerve regeneration, and reduced atrophy (**Fig 6**).

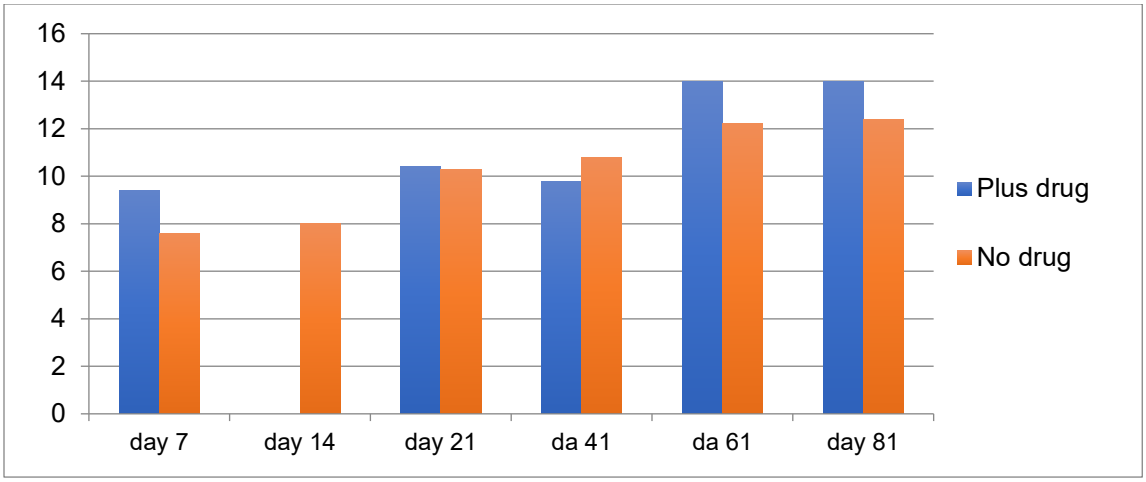


Figure 6. Muscle was cut longitudinally, fixed, and stained with anti-laminin antibody. Data is derived from the analysis of co-apted FDP tendon/muscle with drug (blue) vs co-apted FDP tendon/ muscle without drug (red). The number of samples/timepoint is n=1-2, and the number of fibers per slide counted were n=5-7. The p values for each day were day 7 (p=0.026); day 21 (p=0.9); day 41 (p=0.23); day 61 (p=0.14); and day 81 (p=0.12), with day 7 being significantly different and days 6 and 81 being statistically suggestive. The Y-axis is the width of the fibers and the x-axis is the day of harvest.

In future experiments, we have cut the FDP muscle cross-sectionally and will determine the muscle fiber shape and area. We have also harvested the Flexor digitorum superficialis (FDS) muscle (which derives from the median nerve) which has been cut longitudinally to determine nerve/muscle junction by quantitative NMJ analysis by staining muscle with bungertoxin and neurofilament (NF) to look for interactions.

To examine nerve growth or degeneration, the distal and carpal tunnel nerves will be stained with KI-67 (proliferation) and S100 (glial cells and neurons). Also, the co-aptation (proximal) site nerves have also been stained for inflammation and scar formation with trichrome and PSR and an analysis of macrophage phenotype is being carried out.

For all of these studies in Aim 2, the Giladi laboratory has been consulting with Dr. Tuffaha, originally trained in the Brandacher laboratory (3,4) and now running his own primary laboratory, on fine details of the test procedures, strength testing, and tissue harvest/staining approach.

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What opportunities for training and professional development has the project provided?
Nothing to Report

How were the results disseminated to communities of interest?
Our results were written up and submitted to the MHSRS meeting for presentation. These were accepted for a poster presentation. However, for the second year in a row, the meeting was canceled due to COVID-19. The abstract was published online.

What do you plan to do during the next reporting period to accomplish the goals?
For our first goal, since we saw effects of BMP2 and DPCA on bone volume, we will increase drug delivery to once per month.

For our second goal, we are seeing differences using the co-aptation nerve transection technique. We will explore giving a drug treatment (2 injections on da0 and da 8) once per month.

For the 3rd and 4th goals, ongoing experiments will perfect drug release over a longer period of time. These studies will be done in vitro and in vivo. As our test system, we will use ear hole closure in mice and rats.

The Following Describes Activity in the Messersmith Laboratory at University of California, Berkeley

Aims 1-3. Optimization of DPCA drug delivery system

One purpose of this aim is to supply DPCA drug delivery systems for use in aims 1 and 2. A second purpose of this aim is to improve and optimize the DPCA delivery system.

Synthesis of PEG-DPCA Nanogel for Aims 1 and 2.

We note that reporting on this task is brief and similar to last year because it is mainly a service task for Aims 1 and 2. The Messersmith group's role is to synthesize, package and send units of DPCA nanogel to the Heber-Katz and Giladi labs for use in mouse and rat regeneration experiments. As shown in **Figure 7**, the nanogel is composed of two DPCA-polymer conjugates, P7D3 and P80D6, which in the presence of water self-assemble into nanofibers which entangle to form a gel network. The gel network is shear-thinning, facilitating injection through a needle and syringe into a target tissue. Subsequently, hydrolysis of the DPCA-PEG linker liberates free DPCA to upregulate HIF-1α and enhance regeneration.

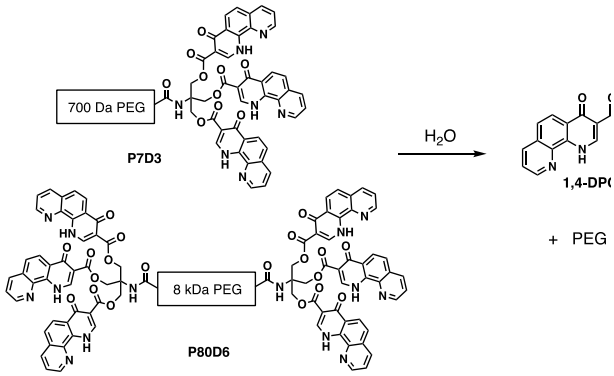

Figure 7. Composition of **P7D3** and **P80D6**. In the presence of water, the ester bond is cleaved to release active DPCA drug.

Improvement and Optimization of the 1,4-DPCA Drug Delivery System. Additional long term objectives of this aim are to develop new administration routes for 1,4-DPCA such as oral or local (e.g. topical) delivery. Summarized below are results obtained during the last year toward these goals.

Bioadhesive Nanoparticle Delivery Systems for 1,4-DPCA.

One of our goals is to develop approaches for localized delivery of DPCA directly to a wound site. Degradable poly(lactic-co-glycolic acid) (PLGA) nanoparticles are an interesting option for this purpose, as they can be loaded with drug and coated with a bioadhesive polymer for retention at the wound site. As can be seen in **Figure 8,** we designed a bioadhesive PLGA nanoparticle that was coated with a mussel adhesive protein-inspired copolymer (PLL-Cat). The copolymer is inspired by the catechol and amine rich proteins found at the mussel byssus-substrate interface, which have evolved to strongly adhere to organic and inorganic surfaces. PLL-Cat was characterized by NMR and UV-vis, confirming the presence of catechol in about 20% of repeat units. PLGA nanoparticles were coated with a thin film of PLL-Cat and the particle size and surface charge (zeta potential) characterized by dynamic light scattering (DLS). The uncoated PLGA nanoparticles averaged 72 nm in diameter, whereas PLL-Cat coated nanoparticles were modestly larger, indicating the presence of the PLL-Cat coating. The presence of the coating was further confirmed by zeta potential measurements, which showed a positive surface charge of PLL-Cat coated PLGA nanoparticles dues to the positive charge associated with the amino groups of the copolymer.

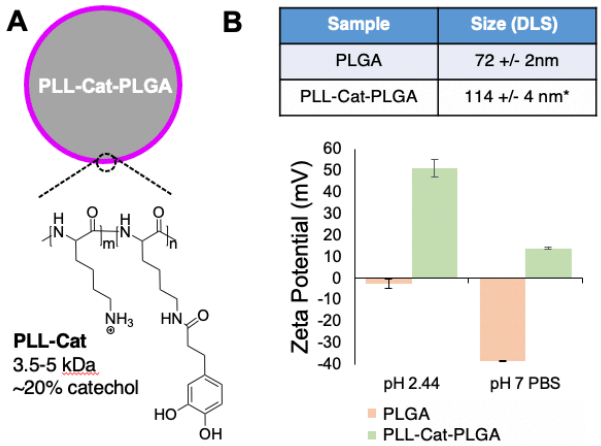

Figure 8. Design of bioadhesive nanoparticles for 1,4-DPCA delivery. A. The nanoparticles are composed of a PLGA core with a surface coating of a mussel-inspired polymer (PLL-Cat). B. DLS and zeta potential analysis show that the particles are in the 70-120 nm size range, and those coated with PLL-Cat possess a significant positive surface charge.

Next, we examined the nanoparticles for ability to adhere to extracellular matrix proteins commonly found on tissue surfaces. Adsorption of PLGA and PLL-Cat coated PLGA nanoparticles on mucin (chosen as a proxy for tissue ECM) was detected using quartz crystal microbalance with dissipation (QCM-D). QCM-D is a sensitive technique for measuring biomolecular adsorption phenomena, as it is particularly sensitive to mass, as indicated by a frequency shift (negative values of Δ (change) frequency are directly correlated to mass adsorbed). Since the nanoparticles have high mass, this technique is well-suited to detect nanoparticle adsorption. As shown in **Figure 9**, introduction of PLL-Cat-PLGA nanoparticles to a mucin coated sensor produced a dramatic change in frequency indicative of strong adsorption, in contrast to weak adsorption of uncoated PLGA nanoparticles. These data confirm that PLGA nanoparticles can be made bioadhesive through introduction of a mussel-inspired polymer coating.

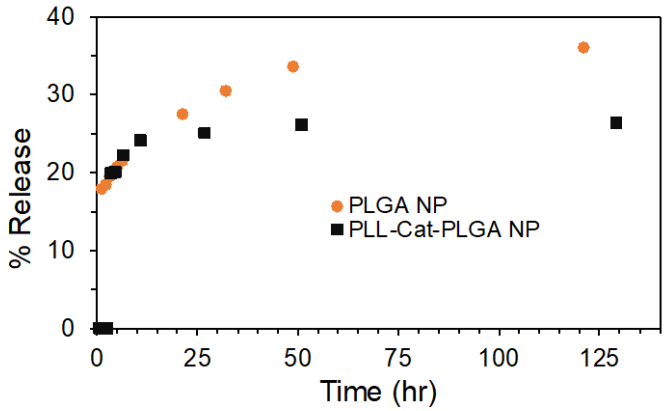

Figure 10. Release of DPCE from PLGA nanoparticles.

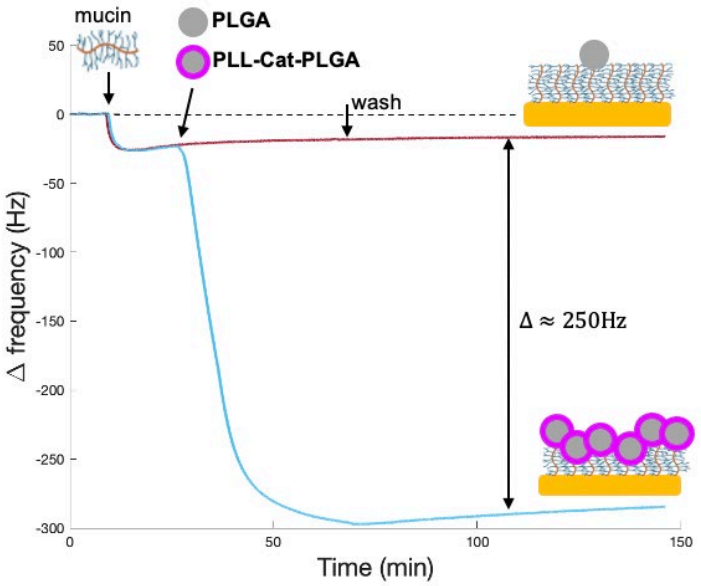

Figure 9. Bioadhesion of nanoparticles using QCM-D. First, a thin film of mucin was adsorbed onto a bare gold sensor, followed by introduction of a PLGA nanoparticle suspension for 30 minutes before washing with buffer. The decrease in frequency observed for PLL-Cat-PLGA nanoparticles is indicative of adsorption to the mucin.

Last year we described the synthesis of 1,4-DPCE, the inactive ethyl ester of 1,4-DPCA. 1,4-DPCE can be considered a prodrug in that it liberates 1,4-DPCA through ester hydrolysis in the presence of water. We recently conducted a comparison of DPCE release from PLL-Cat-PLGA and PLGA nanoparticles. Particles were suspended in buffer and the DPCE content in the buffer was measured by HPLC (**Figure 10**). The results showed similar release rates for the first 10 hours followed by a divergence in DPCE release, with release of DPCE from PLL-Cat-PLGA being slower than from PLGA control.

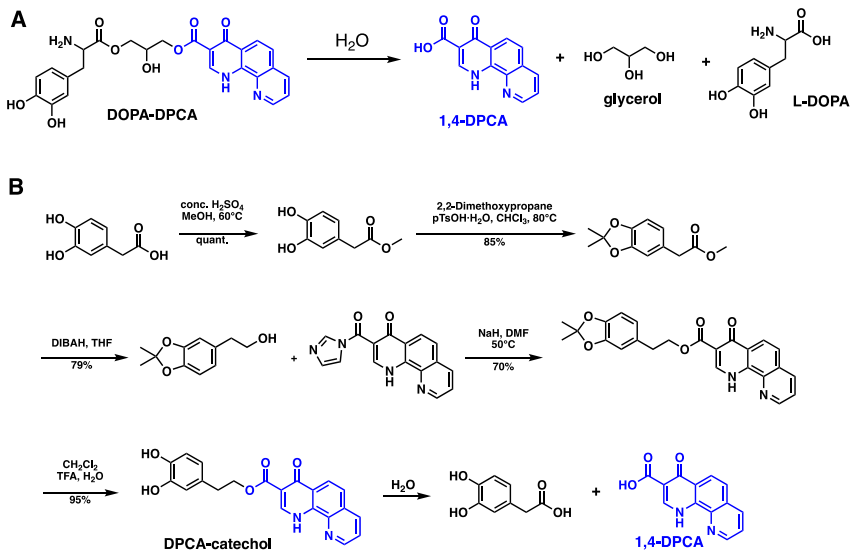

Figure 11. Bioadhesive molecular prodrugs. A. Representative chemical structure of a novel adhesive prodrug target. The prodrug is designed to include an adhesive catechol moiety conjugated to DPCA via a glycerol linker and hydrolysable ester bonds. DPCA is shown in blue. B. Chemical synthesis of DPCA-catechol prodrug.

Finally, we proposed to develop peptide/peptoid prodrugs as alternative delivery systems for DPCA. Our rationale is that a simple small molecule DOPA-DPCA conjugate could be directly administered to a wounded tissue, where it would adhere and release DPCA locally to enhanced tissue regeneration. An example target for this work is a conjugate of an adhesive DOPA peptide with DPCA as shown in **Figure 11A**. We are currently developing the protocols for synthesis of such compounds. As an example of our approach, the synthetic scheme for a related prodrug is shown in **Figure 11B**. This catechol-DPCA compound was successfully synthesized in high yield and will be further tested in the upcoming months.

What opportunities for training and professional development has the project provided?
Nothing to Report

How were the results disseminated to communities of interest?
We published one preprint this year:

DeFrates KG, Engström J, Sarma NA, Umar A, Shin J, Cheng J, et al. The Influence of molecular design on structure-property relationships of a supramolecular polymer prodrug. ChemRxiv. Cambridge: Cambridge Open Engage; 2022.

What do you plan to do during the next reporting period to accomplish the goals?
During the No Cost Extension we will continue to synthesize and provide units of nanogel to the Heber-Katz and Giladi labs to complete the in-vivo digit and nerve studies. Additionally, we will continue the synthesis and in-vitro characterization of bioadhesive nanoparticles and molecular conjugates of DPCA.

4. IMPACT

What was the impact on the development of the principal discipline(s) of the project?
Nothing to Report

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
Nothing to Report

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
Nothing to Report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	Ellen Heber-Katz, Ph.D.
Project Role:	Principal Investigator
Researcher Identifier (e.g., ORCID ID):	
Nearest person month worked:	4 CM
Contribution to Project:	Dr. Heber-Katz oversees all technical, budgetary and reporting aspects of this proposal. Dr. Heber-Katz directly supervise Dr. Azamat Azlanukov in animal studies and gene expression analysis. She also directly supervises Dr. Khamilia Bedelbaeva who carries out histological analysis and immune staining.
Funding Support:	No other funding support

Name:	Azamat Azlanukov, Ph.D.
Project Role:	Research Assistant Professor
Researcher Identifier (e.g., ORCID ID):	
Nearest person month worked:	4 CM
Contribution to Project:	Dr. Azlanukov has expertise in molecular biology as well as animal studies and in using the microCT, IVIS, and FACS analysis and ImageJ Analysis Software. He is responsible for western blotting and QPCR gene expression studies for all experiments.
Funding Support:	No other funding support

Name:	Khamilia Bedelbaeva, Ph.D.
Project Role:	Research Assistant Professor
Researcher Identifier (e.g., ORCID ID):	
Nearest person month worked:	10 CM
Contribution to Project:	Dr. Bedelbaeva is responsible for a wide variety of molecular biology techniques including tissue and cell culture, preparing cells for FACS analysis, preparing all tissue samples for processing of tissues, sectioning, histology, and immunohistochemistry analysis of injured tissue from the Heber-Katz and Giladi studies.
Funding Support:	No other funding support

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

- *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. (Ex. a previously active grant has closed and/or if a previously pending grant is now active.)*

What other organizations were involved as partners?

Nothing to Report

- *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.*

Organization Name:	MedStar Union Memorial Hospital, Curtis National Hand Center
Location of Organization:	6525 Belcrest Rd. Hyattsville, Md. 20782-2003
Partner's contribution to the project:	N/A
Financial Support:	N/A
In-kind support:	N/A
Facilities:	N/A
Collaboration:	N/A
Personnel exchanges:	N/A
Other:	N/A

Organization Name:	University of California, Berkeley
Location of Organization:	2195 Hearst Ave. Berkeley, CA. 94720-1103
Partner's contribution to the project:	N/A
Financial Support:	N/A
In-kind support:	N/A
Facilities:	N/A
Collaboration:	N/A
Personnel exchanges:	N/A
Other:	N/A

8.Special Reporting Requirements

Nothing to report

9.Appendices

Nothing to report