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14. ABSTRACT

In the proposed studies, we are attempting to leverage 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 and nerve injuries and digit regeneration. Here, we are exploring the effect of this drug on 1) digit regrowth post-amputation and nerve growth, 2) the role of peripheral re-innervation in rat forelimbs on injury restoration, and 3) optimization of drug potency and delivery in these systems. Our progress during this first year includes studies on surgically amputated digits in mice using Micro-CT analysis and immunohistochemistry showing changes with drug therapy as early as day 7. Significant changes at the digit cut site and formation of a boney callus are observed. Optimal drug dosing experiments for the rat forelimb nerve repair experiments were accomplished. Finally, we made progress in developing a new carrier system for 1,4-DPCA, making it more potent and easier to deliver. During this coming year, we will explore both earlier and later timepoints post digit amputation, begin extensive studies in forelimb nerve regrowth and effects on digit function, and further develop the 1,4-DPCA drug delivery system and modification of 1,4 DPCA compounds.

15. SUBJECT TERMS

Blastema, Bony callus, Digit amputation, HIF-1α, Micro-CT, Marrow canal, Neurofilament, Nerve regeneration, PEG-DPCA-nanogel, Sprague Dawley rats, Swiss Webster mice

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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 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 rat model 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

Blastema Formation, B6/129 Female Mice, Digit Amputation, Grip Strength Analysis, Inflammation, Joint Formation, Micro-CT Analysis, Nerve Regeneration, Picro-Sirius Red

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 #3. We initiated a second group of studies (Exp #3; Exps #1 and 2 were presented last year) to determine the importance of the site of drug injection on drug-induced digit regeneration and nerve regrowth in 2 month old B6/129 male mice. Again, the second phalanx of the middle digit from the hind-paw was surgically amputated midway between the proximal and distal joints under isoflurane and buprenorphine administered every 8 hours. (see **Fig 1**). We used 2 mice/group and the groups consisted of 1) mice not given drug (control); 2) mice given drug in the foot for local treatment; and 3) mice given drug subcutaneously (SQ) distal to the wound and thus systemically. These mice were followed for 6 months using microCT analysis after receiving one round of DPCA-PEG, unlike our initial experiments which lasted approximately 1 month. At this time, we carried out immunohistochemistry to examine nerve, tendon, and bone growth (**Fig 2**).

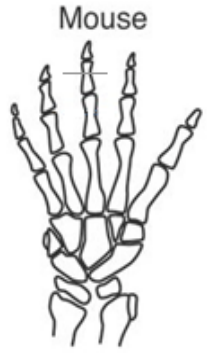


Figure 1

I. Histological Changes after Drug Administration

In these sections we used digit sections from mice that had undergone digit amputation 6 months earlier. We compared H&E sections to sections stained with anti NF (nerve fibers) and ant-Gap 43 (nerve endings) antibodies and found several striking features. In particular, only in the mice given drug SQ, we noticed a circular staining pattern that looked very similar to that of a joint potentially forming at the cut end (**Fig 2**). A comparison to a preformed joint shows that the structure has a very similar shape.

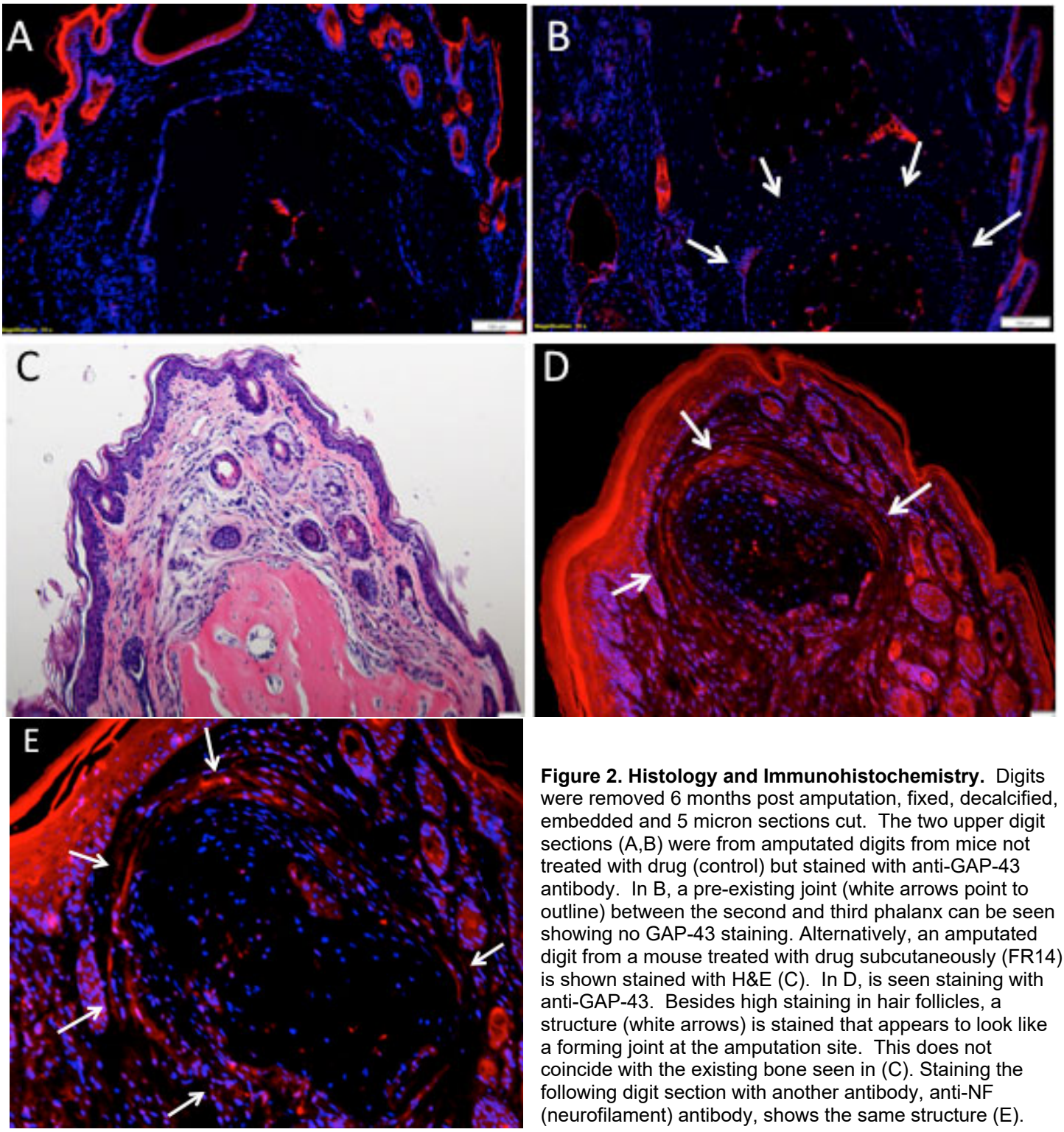


Figure 2. Histology and Immunohistochemistry. Digits were removed 6 months post amputation, fixed, decalcified, embedded and 5 micron sections cut. The two upper digit sections (A,B) were from amputated digits from mice not treated with drug (control) but stained with anti-GAP-43 antibody. In B, a pre-existing joint (white arrows point to outline) between the second and third phalanx can be seen showing no GAP-43 staining. Alternatively, an amputated digit from a mouse treated with drug subcutaneously (FR14) is shown stained with H&E (C). In D, is seen staining with anti-GAP-43. Besides high staining in hair follicles, a structure (white arrows) is stained that appears to look like a forming joint at the amputation site. This does not coincide with the existing bone seen in (C). Staining the following digit section with another antibody, anti-NF (neurofilament) antibody, shows the same structure (E).

II. Bone changes after amputation using Micro-CT Scanning

To look for changes in bone structure over time**,** we carried out Micro-CT scanning of all mice for up to 6 months. From these, we determined changes in bone length, bone density, and bone volume. In Figures 3 through Figure 8 are shown one control mouse (FA4, no drug, the second mouse died) and two mice given

drug in the foot near the amputation site (FK4, FK14); and two mice given drug SQ systemically (FG4, FR14). From this study, we observed multiple differences between the experimental and control animals.

Changes in Bone Length: Measurements of bone length from Micro-CT images were carried out. In **Fig 3** is shown changes in bone length, with the largest amount of growth in the SQ injected mice. It was noted that after amputation all groups showed bone degeneration for approximately one month. At that point, regrowth begins to be seen.

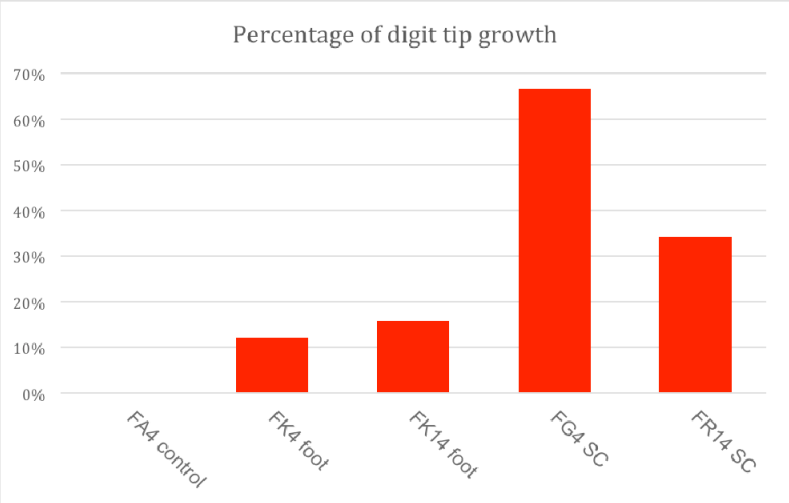


Fig 3. Changes in Bone Length. Bone length changes after the degenerative response and then until 6 months after amputation.

Changes in Bone Density: Measurements in bone density were also carried out. The most striking aspect of this is the variation over time in all mice and no significant changes between groups. (**Fig 4**).

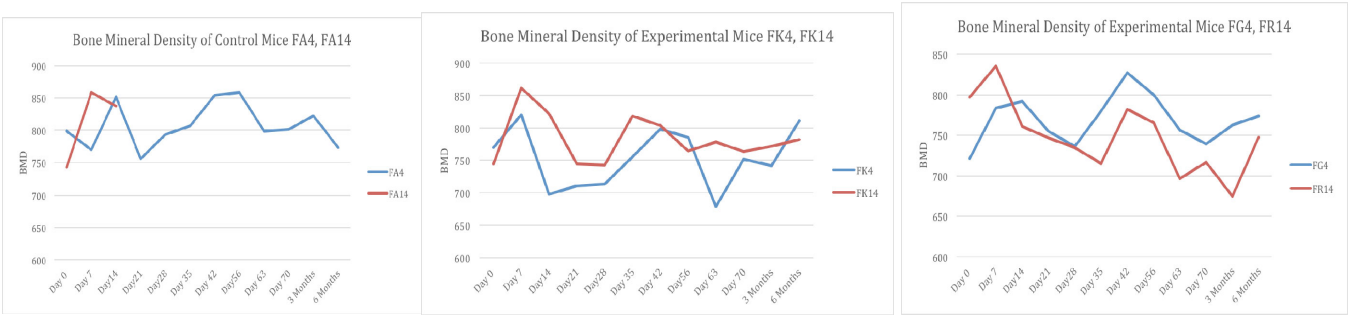


Fig 4. Changes in bone density. The left panel shows the controls, the middle panel shows variation in foot-injected mice, and the right panel shows similar variation in the SQ-injected mice.

Changes in Bone Volume. Measurements in bone volume show differences between all groups. Changes seen over time suggest further intervening drug injections.

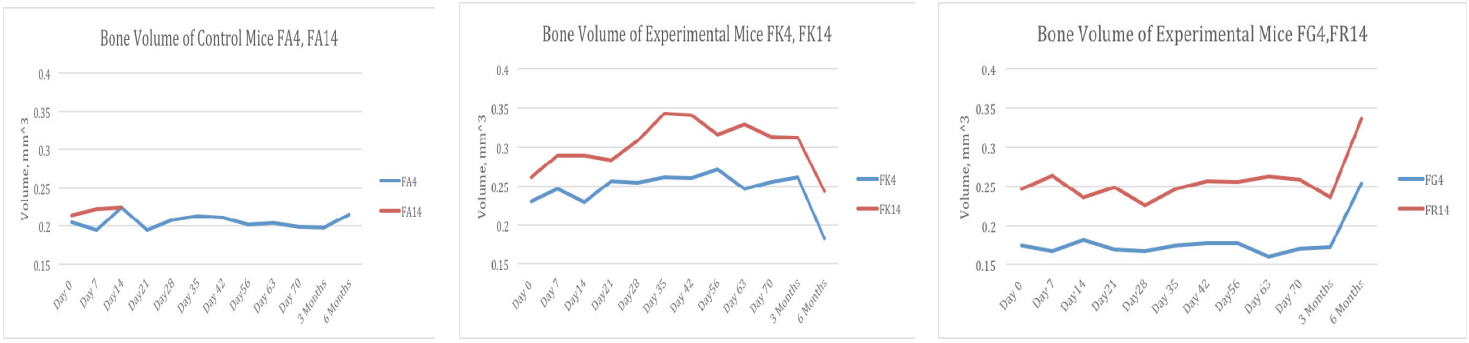


Fig 5. Changes in bone volume. The control (left panel) shows no changes over time, the foot-injected mice show the largest increases but falls precipitously at 6 months (middle panel), and the SQ-injected mice (right panel) show increases at 6 months.

Micro CT images from each treatment group.

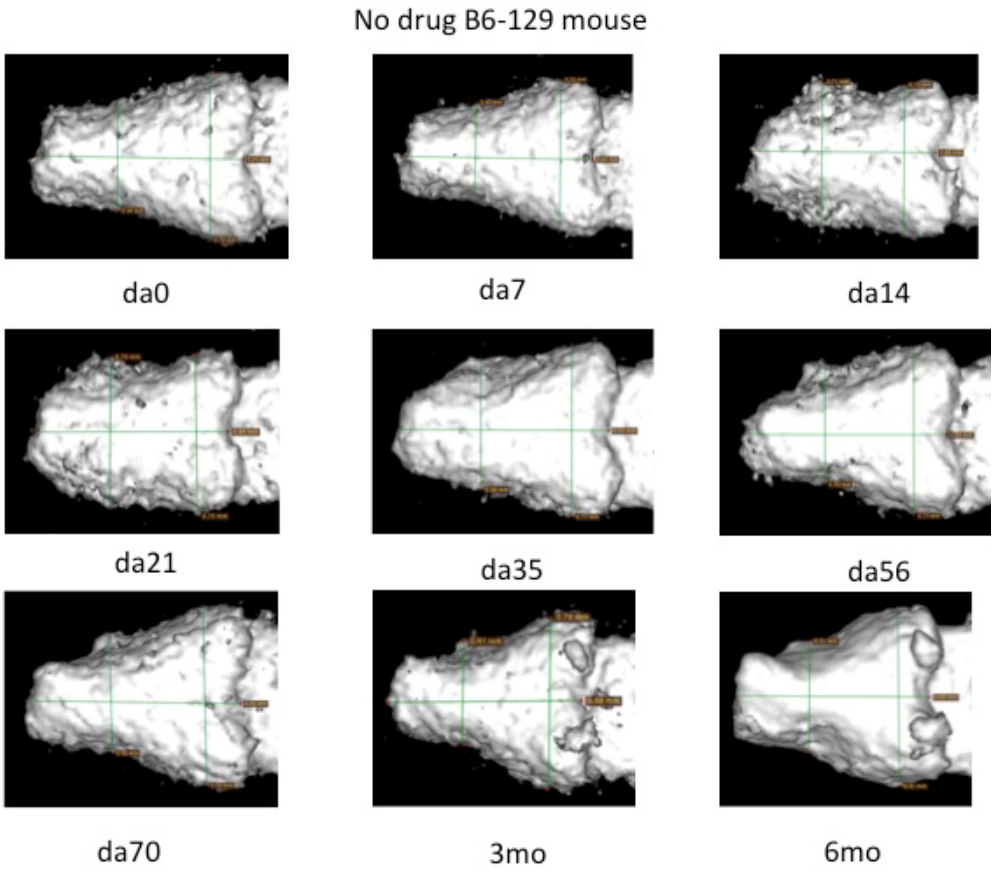


Fig 6: Micro-CT images of digits post amputation without drug treatment. Scans of an amputated digit are shown for a control mouse over a 6 month period. There is a boney callus formed at 21 days, reported previously, but then it takes on its original shape.

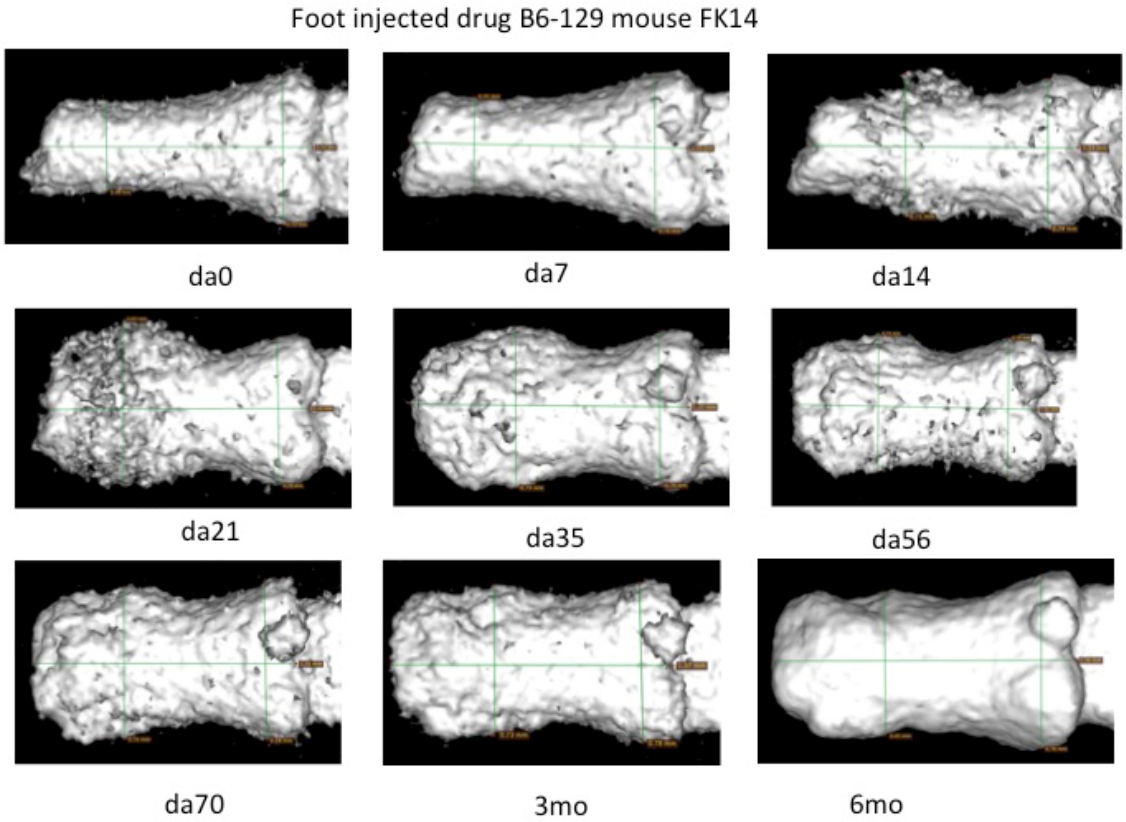


Fig 7: Micro-CT images of digits post amputation with drug injected in the foot near the amputation site. Scans of an amputated digit are shown for a foot-injected mouse over a 6-month period. There is a boney callus formed which, unlike the control) does not disappear and the callus adds to the length of the growing digit.

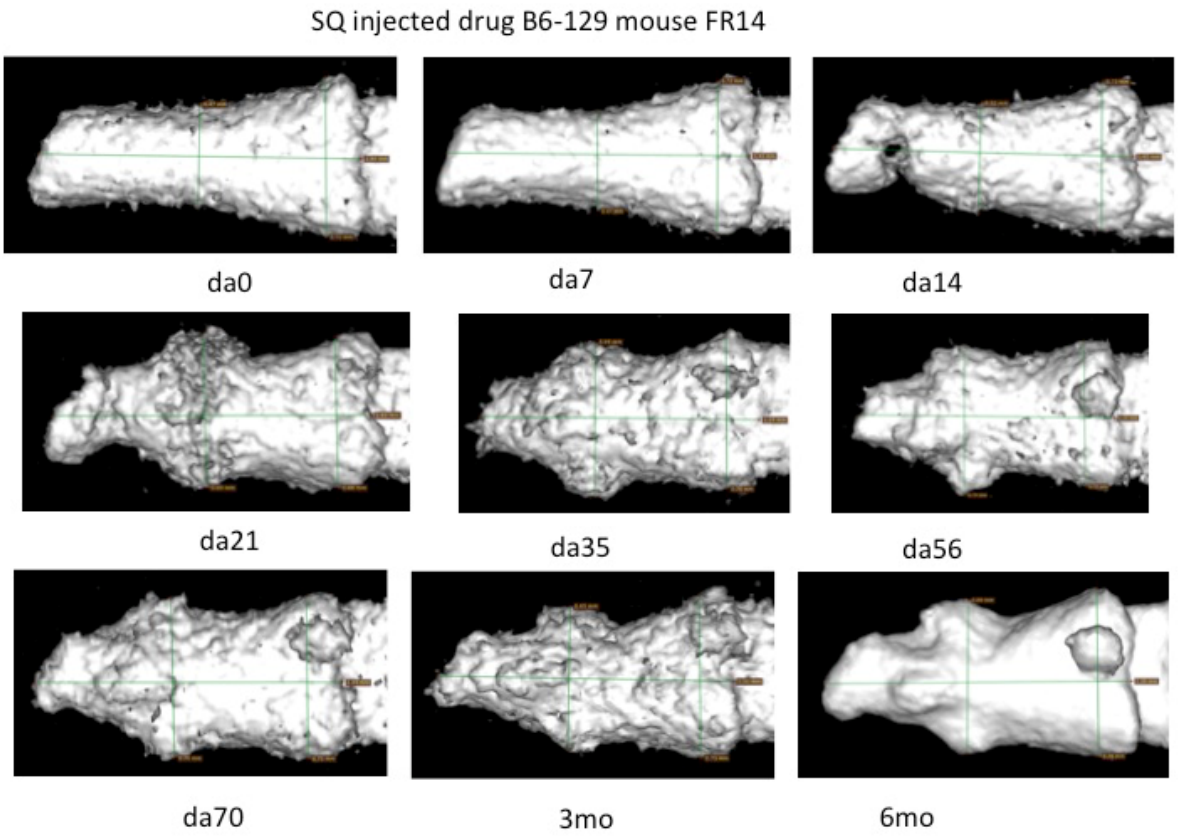


Fig 8: Micro-CT images of digits post amputation with SQ/systemic drug treatment. Scans of amputated digits are shown for a mouse injected SQ with drug delivered systemically over a 6 month period. There is a boney callus formed at 21 days, reported previously. However, a novel feature here is the remodeling seen with something resembling a forming joint. It is in histological sections, that we see nerve staining around a structure that again looks like a forming joint (Fig 2D,E).

Studies in our laboratory on aging mice with osteoporosis have shown that one injection round (day 0, day 8) with drug leads to changes from osteoporotic, to osteopenic to normal bone density over time. These changes last for approximately 3 months (**Fig 9**). Thus, a second round of injection after 3 months might be necessary and we will follow these results in our next digit experiments. Also, it has been reported that BMPs affect bone growth and joint formation. We will also test BMPs near the injury site (ref 2).

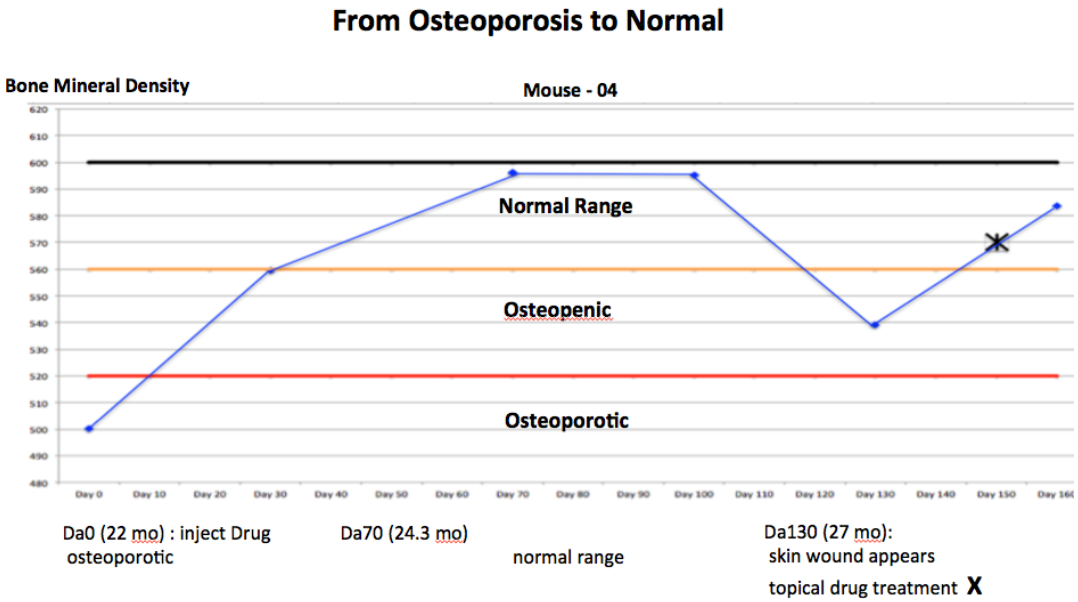


Fig. 9. Aged Mouse shows Reversal of Osteoporosis after Drug Administration. At day 70 after drug administration (day 0, day 8), the mouse femur was imaged by micro CT and bone density measured. The osteoporotic range is the red line and below, the osteopenic range is between the yellow and red line, and the normal range is the yellow line and above.

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

Here we present the results from the initial testing groups. Our goal was to determine the effect of drug treatment on nerve regeneration after nerve coaptation. By measuring grip strength, we tracked nerve recovery in the rats. Subgroups 1 (repair) and 2 (no repair) served as dose efficacy validation prior to deployment in the larger groups. In subgroup 1, the median and ulnar nerves were cut and repaired (co-apted) via an ulnar to median nerve transfer. Originally, in subgroup 2, 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. It should be noted that in the "no-repair" subgroup reported on in this write-up, 3/10 of the mice did regain grip strength due to this suture loosening issue; we have excluded them from the data. In the group we are currently testing but is not documented in this write-up, we have switched to the 4 stump suture technique and thus far no mice have regained grip strength. Moving forward we will use this technique for the control groups.

Subgroup 1 and 2 both consisted of 10 rats, with 5 rats receiving drug treatment (1,4-DPCA) post-operatively on Day 0 and at Day 8. Animals were tested every 7 days for regain of grip strength. 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 shape 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 3 trials was recorded. Baseline measurements were taken 1-3 days pre-operatively and every 7 days post-operatively until Day 98.

Four parameters were compared based on grip strength:

1. No DPCA treatment: Sutured to muscle (no repair) vs. Co-apted (repaired) (Figure 10A)
2. DPCA treatment: Sutured to muscle vs. Co-apted (Figure 10B)
3. Sutured to muscle: DPCA treatment vs. no DPCA treatment (Figure 10C)
4. Co-apted: DPCA treatment vs. no DPCA treatment (Figure 10D)

1. Comparing groups, the animals with co-apted (repaired) nerves showed quicker recovery and increase of grip strength compared to nerves sutured to muscle (no repair). Co-apted animals regained grip strength at approximately Day 35.

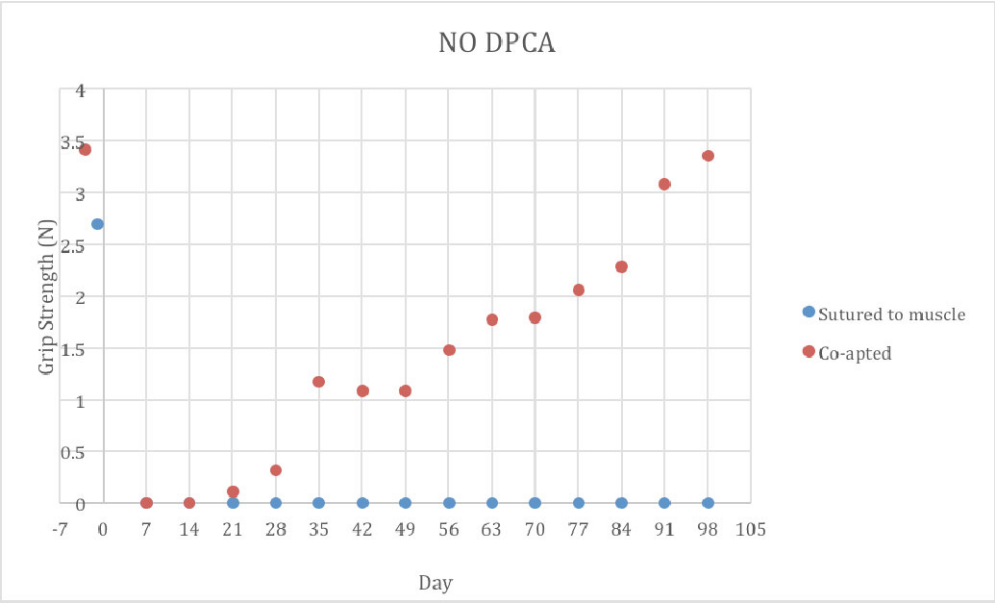


Figure 10A: Grip strength data of No DPCA treatment groups: Co-apted vs. Sutured to muscle.

In the suture to muscle (no repair) group, 2 animals regained grip strength around Day 63 as a sutured nerve came away from the muscle and self-copated. From here we changed the technique which was described earlier in the report. Those two animals have been excluded from the analysis.

2. Comparing groups, the animals with coapted nerves showed quicker recovery and increase of grip strength compared to nerves sutured to muscle (no repair). Animals with coapted nerves regained grip strength around Day 28. One animal was excluded from analysis as it displayed a sudden decrease in grip strength at day 91 (unknown reason) and is not representative of the population.

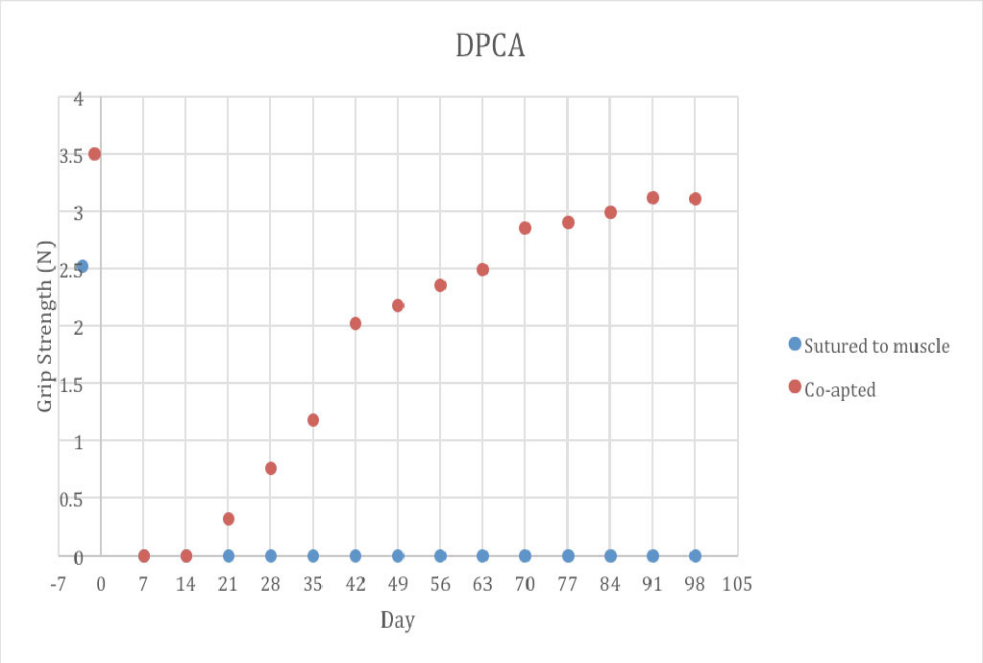


Figure 10B: Grip strength data of DPCA treatment groups: Coapted vs. Sutured to muscle.

3. Both DPCA and No DPCA groups displayed no grip strength for the full 98 days. In this group, 2 animals regained grip strength around Day 63 as a sutured nerve came away from the muscle and was able to reconnect on its own. From here we changed the technique which was described earlier in the report. Those two animals have been excluded from the analysis.

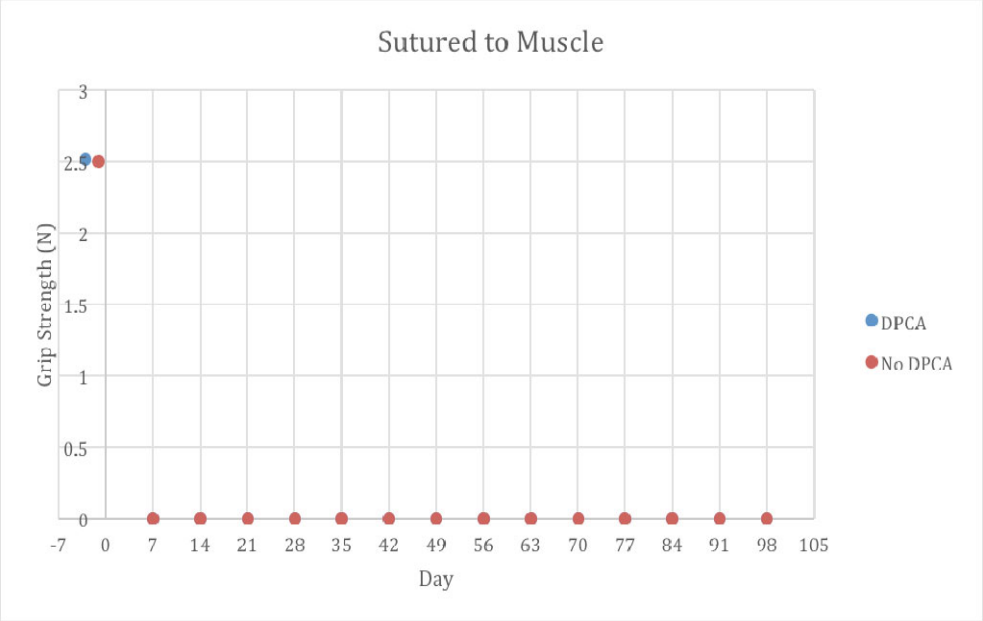


Figure 10C: Grip strength data of sutured to muscle groups: DPCA vs. No DPCA.

4. Comparing groups, the animals with coapted nerves and with DPCA showed quicker recovery and increase of grip strength compared to No DPCA. One animal was excluded from analysis as it displayed a sudden decrease in grip strength at day 91 and is not representative of the sample population.

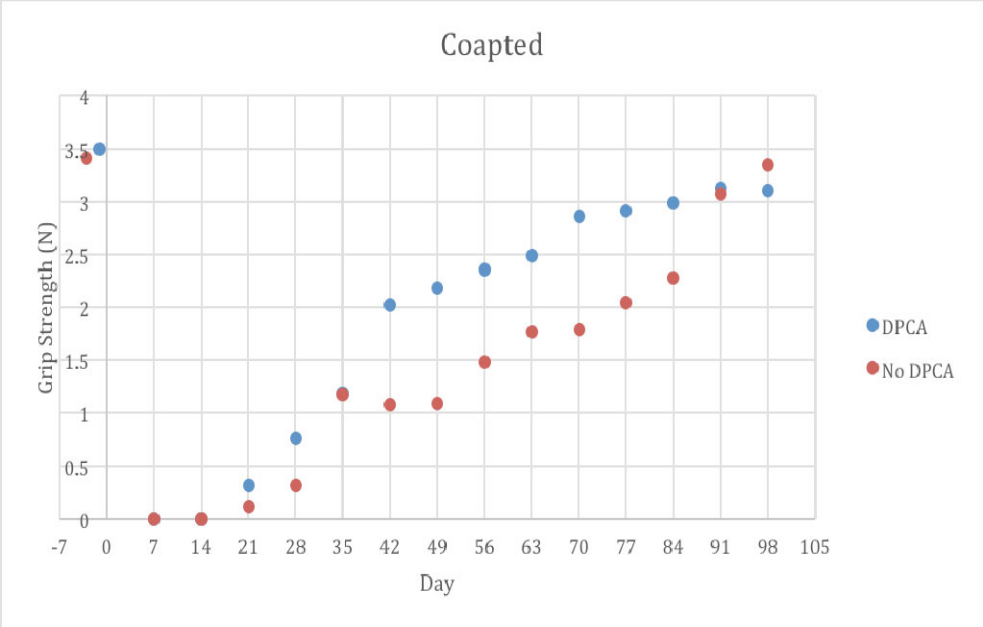
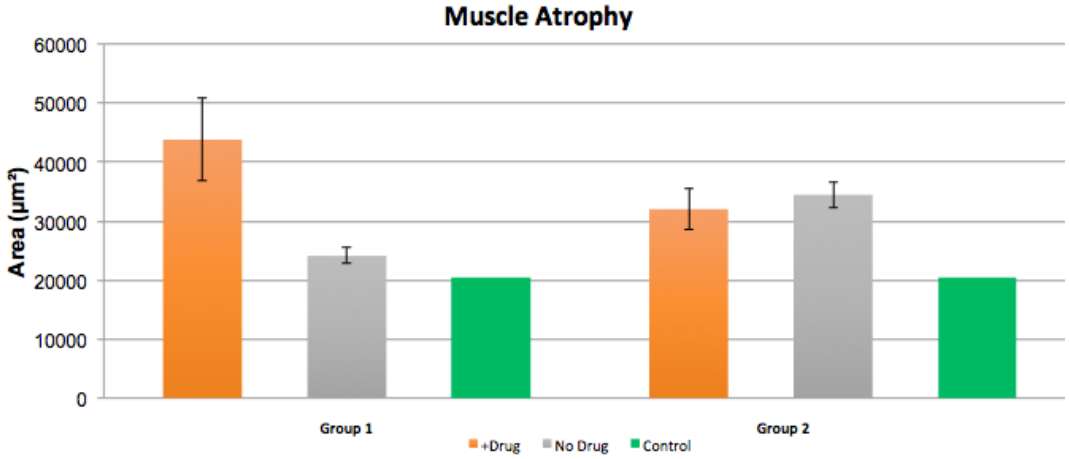


Figure. 10D: Grip strength data of co-apted groups: DPCA vs. No DPCA

Five and twelve weeks after surgery, tissue sections from the surgical site were recovered. The sections were cut into 3 parts. Tissue was sent to Philadelphia for the Heber-Katz laboratory to carry out histochemistry and immunohistochemistry for nerve growth, muscle degeneration, and scar formation.

To examine the nerve/muscle junction, local muscle adjacent to the injury site was removed, and we have begun to examine quantitative NMJ analysis by staining muscle with bungertoxin and neurofilament (NF). To look at muscle atrophy, we stained muscle sections with anti-laminin antibody and counted the number of muscle fibers/given area. As seen below, Group 1 (co-apted nerve) showed clear differences between rats treated with or without drug. Those animals given drug showed increased muscle fiber size, increased regeneration, and reduced atrophy. The control is the value seen in normal rats. In Group 2 (sutured to muscle), there is little difference between rats treated with or without drug, similar to what was seen with grip strength. However, in this group we are seeing muscle size bigger than the normal control. (**Fig 11**)



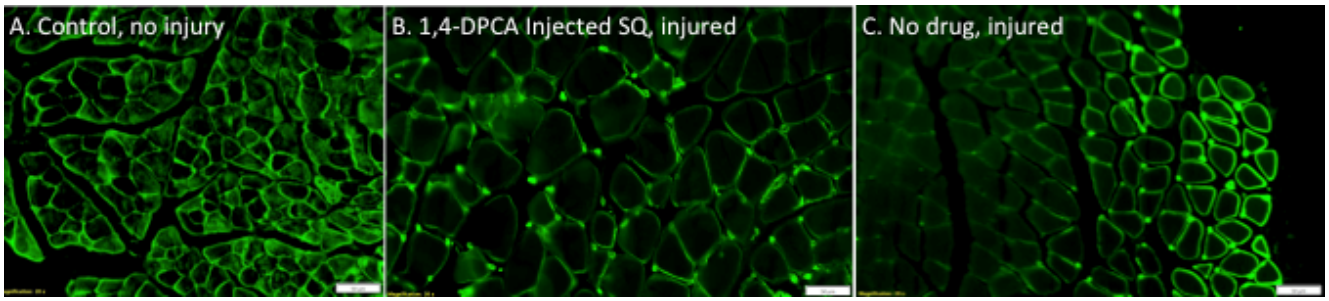


Figure 11. Muscle was cut transversally, fixed, and stained with ant-laminin antibody. Data is derived from analysis of Group 1 (co-apted, left), Group 2 (sutured to muscle, right) and un-surgerized rats (control). Above, are images of muscle fibers stained with anti-laminin antibody: A) from an uninjured rat; B) from a rat first injured and then given drug on days 0 and 8; and C) from a rat injured but given no drug. The measuring scale bar represents 50uM. For Group 1, the difference between drug and no drug is p=0.05; for Group 2, drug vs no drug is p=0.799.

To examine nerve growth or degeneration, the down-stream (distal) nerve will be stained with KI-67 (proliferation) and S100 (glial cells and neurons). To examine the number of nerve fibers/section and the myelin sheath, instead of using Osmium tetroxide which is very toxic to humans, we have been using the Kluver-Barrera method (**Fig 12**). The results show an increased number of fibers in Group 1 (co-apted) with drug treatment but more fibers without drug in Group 2 (suture to muscle), consistent with grip strength and muscle fiber area. The images below show that the uninjured control (A) has fibers that are large (70%), medium (15%), and small (15%) based on diameter. The drug-treated mouse (B) has medium sized fibers, and the no drug but injured mouse (C) had small sized fibers.

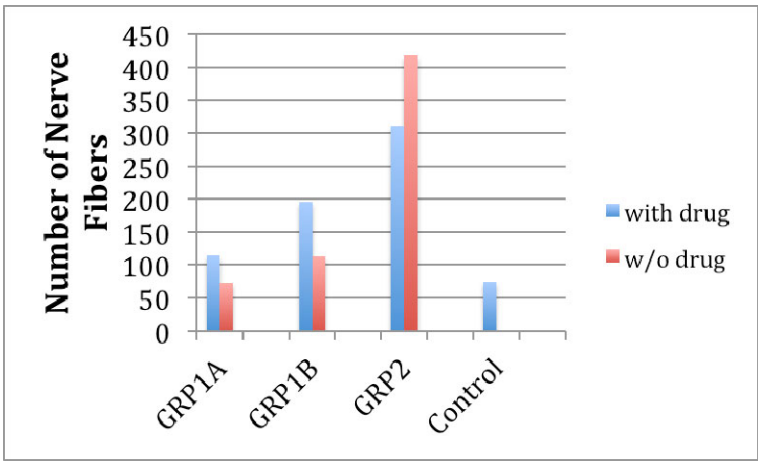
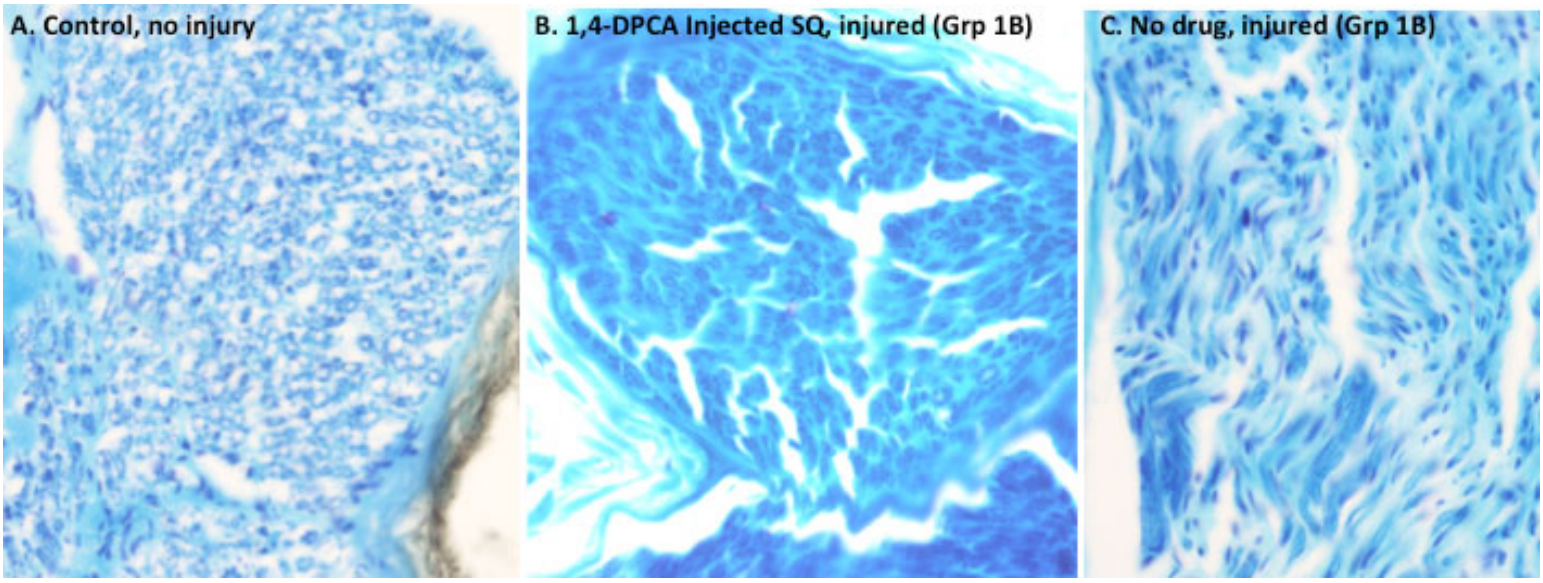


Figure 12. NERVE FIBERS. Distal nerve was fixed and cross-sections were cut in paraffin. The slides were then stained using the Kluver-Barrera method (Luxol Fast Blue). Statistical analysis of the number of fibers per given area for drug vs no drug showed p=0 for Group 1a, p=0.140 for Group 1b, and p=0.04 for Group 2.



The co-aptation (proximal) site nerves are being stained for inflammation and scar formation with trichrome and PSR and an analysis of macrophage phenotype (**Fig 13**).

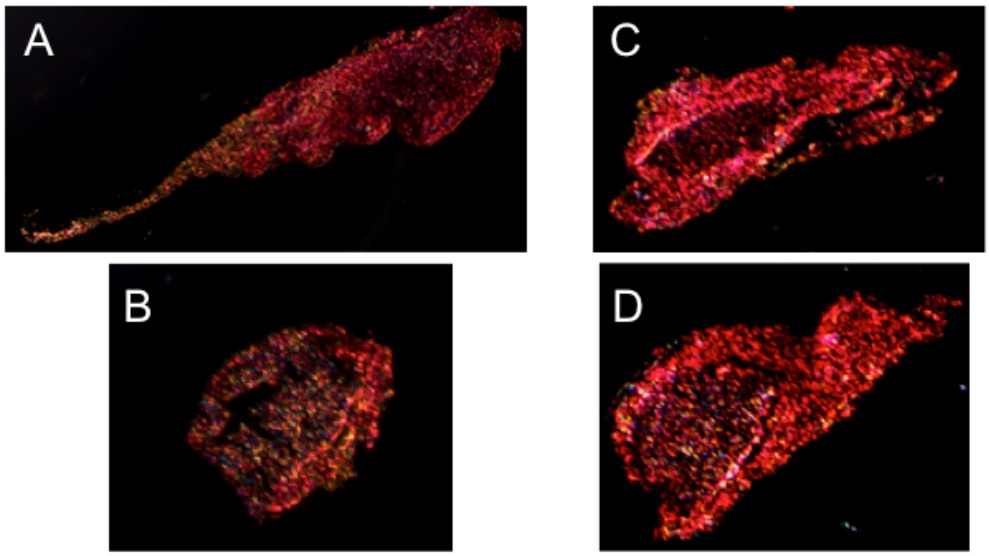


Figure 13. Proximal nerve was fixed and cross-sections cut in paraffin. Sections were stained with Picro Sirius Red (PSR). An example of an injured proximal nerve from two mice treated with drug can be seen in A and B; an example of injured proximal nerve from to mice without drug can be seen in C and D. A,B show a higher level of green fibers indicating Collagen type I. C,D show more red/yellow areas indicating Collagen type III. Images will be analyzed used ImageJ.

For all of these studies in Aim 2, the Giladi laboratory has been consulting with Dr. Tuffaha of the Brandacher laboratory (ref.3-4) who has guided us through the histological analysis.

From the studies above, our initial surgical technique (co-apted) responded positively to drug. This was shown using various healing phenotypes. Using a new “unrepaired” technique of suturing the proximal stumps of the median and ulnar nerves to nearby muscles to prevent any chance of unintentional reconnection, 3 out of 10 rats regained strength. These rats are due for sacrifice by the beginning of September. However, in this case, drug had a negative effect. From our previous studies, this indicates drug toxicity and suggests the need to use lower doses of drug. We will titrate the drug concentration in our next experiments.

The Following Describes Activity in the Messersmith Laboratory at University of California, Berkeley
Aim 3/4 Optimization of nerve regrowth induced by drug/hydrogel

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.
Although this task is critical for success of the overall project, the reporting on this task will be brief because it is mainly a service task for Aims 1 and 2, and much of it has been described in our previous report. 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 14**, 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.

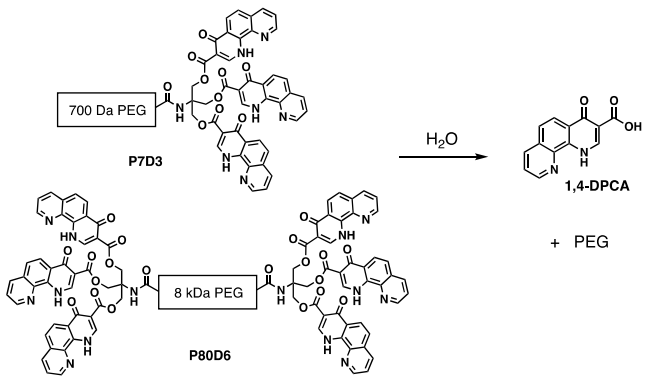


Figure 14. Composition of **P7D3** and **P80D6**. In the presence of water, the ester bond is cleaved to release active DPCA drug.

Improvement and Optimization of the 1,4-DPCA Drug Delivery System.
Additional long term objectives of this aim are to optimize the release rate of DPCA and 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.

Microparticle Delivery Systems for 1,4-DPCA.
Last year we reported our initial efforts to develop poly(lactic-co-glycolic acid) (PLGA) polymer microparticles for sustained release of 1,4-DPCA. We concluded that the solubility properties of 1,4-DPCA make it unsuitable for high efficiency loading into PLGA microparticles due to solvent immiscibility. As an alternative, we synthesized the ethyl ester of 1,4-DPCA (1,4-DPCE). 1,4-DPCE can be considered a prodrug in that it liberates 1,4-DPCA through ester hydrolysis in the presence of water (**Figure 15**). 1,4-DPCE has an improved solubility profile that enhances our ability to prepare high drug loadings in various microparticle systems we are developing. PLGA microparticles with entrapped 1,4-DPCE can be facilely prepared as we reported last year. Furthermore, 1,4-DPCE can be crystallized which affords new opportunities for delivery as described below.

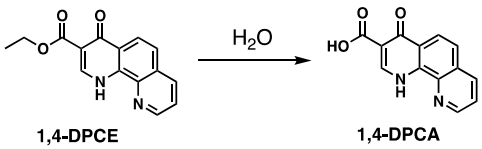


Figure 15. Chemical structure of 1,4-DPCE, a prodrug that hydrolyzes to liberate the active drug 1,4-DPCA.

Bioadhesive Coatings for 1,4-DPCA Delivery.
A desired feature of these microparticles for oral or local delivery of 1,4-DPCA or 1,4-DPCE is tissue adhesion. Microparticles for delivery into a wound site should be tissue adhesive in order to prevent microparticle migration and to localize drug release to the intended location. To enhance bioadhesion, we

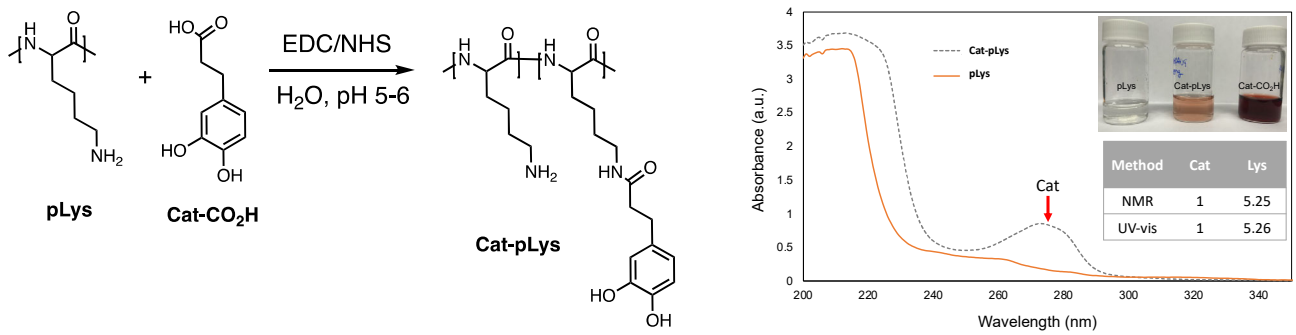


Figure 16. Synthesis and characterization of DOPA-pLys. Shown on the left is the synthetic approach involving conjugation of a catecholic acid to pLys using EDC/NHS chemistry. Shown on the right are characterizations of Cat-pLys. Analysis by UV-vis shows emergence of a 280nm peak indicative of catechol. The inset photo shows the orange color characteristic of catechols treated with Arnow stain. NMR and UV-vis analyses reveal about 1 Cat for every 5 Lys residues.

prepared PLGA microparticles with a bioadhesive coating inspired by mussel adhesive proteins. For attachment to wet surfaces, mussels secrete specialized protein glues that are rich in lysine (Lys) and the unusual amino acid 3,4-dihydroxyphenylalanine (DOPA). The adhesive component of DOPA is the 3,4-dihydroxyphenyl side chain, known as catechol (Cat). Native mussel adhesive proteins and synthetic polymers containing DOPA/Cat and Lys have been shown to be highly adhesive to tissue surfaces. We therefore synthesized a Cat-modified lysine polymer mimic of mussel adhesive protein (Cat-pLys) to be applied to 1,4-DPCA/1,4-DPCE microparticles as a bioadhesive coating (**Figure 16**). Cat-pLys was characterized by NMR, UV-vis and Arnow stain (a catechol-sensitive colorimetric dye) confirming the presence of catechol at a ratio of 1:5.25 (Cat:Lys).

To evaluate bioadhesion, PLGA microparticles were created by a single emulsion technique and Cat-pLys coated on the surface by adsorption from solution. Mucoadhesion was evaluated by a high throughput technique in which a microparticle suspension was added to a microplate well containing adsorbed mucin as a simple mimic of a mucosal tissue surface. After exposure for several hours, the plate was inverted and spun in a centrifuge to remove unattached particles by centrifugal force. Subsequently the remaining attached particles could be quantified by image analysis using ImageJ. The results (**Figure 17**) showed that unmodified PLGA microparticles were not adhesive to the mucin surface whereas pLys coated particles showed enhanced mucoadhesion. However, the highest mucoadhesion was observed for Cat-pLys coated microparticles, demonstrating the adhesive nature of Cat-pLys coated PLGA microparticles.

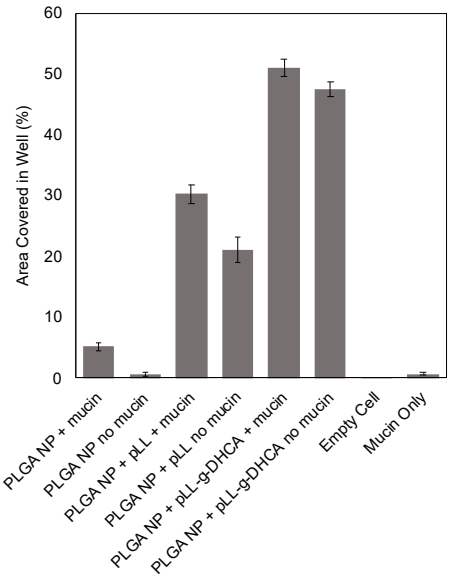


Figure 17. A mucoadhesion assay showed that a Cat-pLys coating dramatically improved PLGA microparticle mucoadhesion compared to uncoated or pLys coated microparticles.

A second approach to improving bioadhesion and altering release kinetics of 1,4-DPCA/1,4-DPCE is to directly coat drug crystals with a bioadhesive coating for use as a topical or oral delivery system. For this purpose we utilized a mussel-inspired catecholamine coating called polydopamine (PDA), which deposits spontaneously from aqueous solution as a conformal coating on surfaces (Messersmith et al., Science, 2007). We crystallized 1,4-DPCE crystals and deposited a thin coating of PDA by immersion for up to 24 hours in an alkaline aqueous solution of dopamine.HCl. 1,4-DPCE crystals were found to be coated with a thin PDA film, thus providing a potential tissue adhesive surface to the crystals (**Figure 18**). Interestingly, we discovered that the PDA coating provided a tool for altering drug release from the crystals of 1,4-DPCE (**Figure 19**).

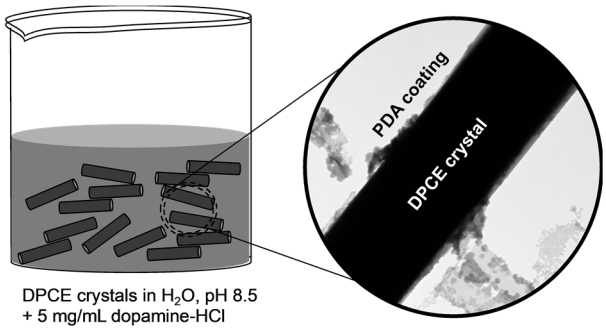


Figure 18. A mucoadhesion assay showed that a Cat-pLys coating dramatically improved PLGA microparticle mucoadhesion compared to uncoated or pLys coated microparticles.

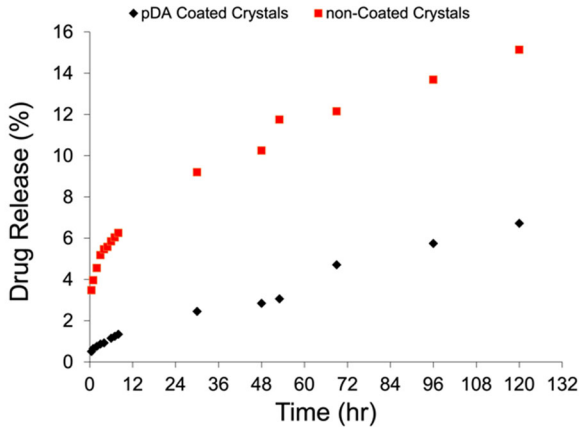


Figure 19. In-vitro drug release studies showed that a PDA coating altered the kinetics of drug release from 1,4-DPCE crystals.

Finally, we recently started developing a new method for making bioadhesive nanoparticles which we anticipate will provide another interesting option for delivery of 1,4-DPCA. The new approach involves the synthesis of block copolymers that self-assemble into nanoparticles with a hydrophobic core and a surface of catechol and amine for bioadhesion (**Figure 20**). We expect that the hydrophobic core can be loaded with drug for topical or oral delivery of 1,4-DPCA.

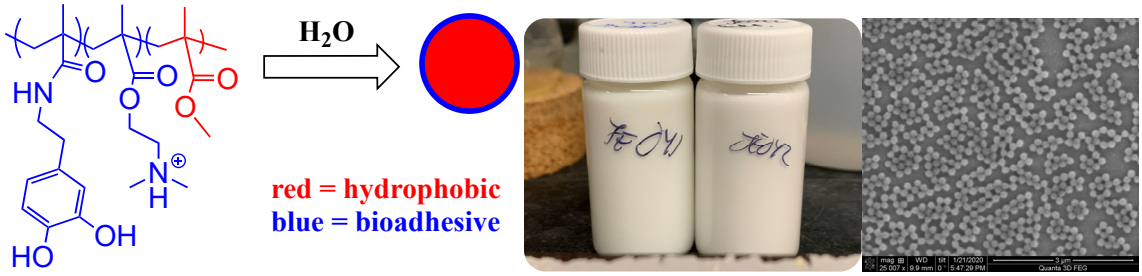


Figure 20. Block copolymers for self-assembly into mucoadhesive nanoparticles. The blue structures are catechol and amine mimics of mussel adhesive proteins and are water soluble, whereas the red portion is hydrophobic. In water, the polymer forms nanoparticles with a hydrophobic core (red) and a bioadhesive surface (blue). These nanoparticles are stable in suspension as shown in the middle photograph, and are composed of ~220nm particles as shown in the electron micrograph at right.

References:

1. Cheng J, Amin D, Latona J, Heber-Katz E, Messersmith PB. 2019. Supramolecular Polymer Hydrogels for Drug-Induced Tissue Regeneration. ACS Nano. 13(5):5493-5501. .PMID: 31067407.
2. 2. Yu L, Dawson LA, Yan M, Zimmel K, Lin Y-L, Dolan CP, Han M, Muneoka K. 2019. BMP9 stimulates joint regeneration at digit amputation wounds in mice, Nature Communications. 41467: 8278-4.
3. Tuffaha SH …Brandacher G. 2016. Growth Hormone Therapy Accelerates Axonal Regeneration, Promotes Motor Re-innervation, and Reduces Muscle Atrophy following Peripheral Nerve Injury. Plast. Reconstr. Surg. 137: 1771.
4. Kern B, Budihardjo JD, Mermulla S, Quan A, Cadmi C, Lopez J, Khusheim M, Xiang S, Park J, Furtmüller GJ, Sarhane KA, Schneeberger S, Lee WP, Hoke A, Tuffaha SH, Brandacher G. 2017. A Novel Rodent Orthotopic Forelimb Transplantation Model That Allows for Reliable Assessment of Functional Recovery Resulting From Nerve Regeneration. Am J Transplant. 17:622-634.

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.

We published one peer-reviewed paper earlier this year:

K. DeFrates, E. Heber-Katz, P.B. Messersmith, "Achieving Regeneration in Mammals through Manipulation of Cellular Oxygen-Sensing Pathways", *Biomaterials* 2021, 269, 120646. PMCID: PMC8279430

What do you plan to do during the next reporting period to accomplish the goals?
For our first goal, we are seeing changes in bone length 6 months from amputation after giving two rounds of drug SQ. We need to increase drug delivery to once per month. Furthermore, we would like to supplement the digit with various BMPs.

For our second goal, we are seeing differences using one transection technique but not another. We will also explore giving drug once per month. We need to perfect the tissue analysis for these studies.

For the 3rd and 4th goals, we plan to perform more detailed in-vitro experiments of 1,4-DPCA and 1,4-DPCE drug release from coated drug crystals and PLGA microparticles coated with bioadhesive Cat-pLys and PDA polymers. The upregulation of HIF-1α in cells exposed to 1,4-DPCE will be characterized in-vitro, as this compound has not been previously reported as a modulator of HIF-1α. More detailed mucoadhesion experiments will be performed to determine the effect of bioadhesive polymer coating on tissue adhesion. Finally, we plan to further characterize the self-assembling block copolymer system for ability to entrap and release DPCA, and to assess its mucoadhesive properties.

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 Repport

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:	Phillip B. Messersmith, Ph.D.
Project Role:	Principal Investigator
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	1.0
Contribution to Project:	Dr. Messersmith oversees all technical, budgetary and reporting aspects of this proposal. Dr. Messersmith also directly supervises other personnel working on the project.
Funding Support:	N/A

Name:	Kyueui Lee, Ph.D.
Project Role:	Postdoctoral Fellow
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	6
Contribution to Project:	Dr. Lee participated in characterization of polymer conjugates of 1,4-DPCA.
Funding Support:	Dr. Lee resigned his position and left the university.

Name:	Joakim Engstrom, Ph.D.
Project Role:	Postdoctoral Fellow
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	11
Contribution to Project:	Dr. Engstrom participated in synthesis of 1,4-DPCA and polymer conjugates of 1,4-DPCA.
Funding Support:	The rest of Dr. Engstrom's support came from faculty discretionary funds

Name:	Arianna Avellan
Project Role:	Graduate Student
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	5
Contribution to Project:	Ms. Avellan participated in preparation of drug crystals, coating drug crystals with bioinspired polymer, and drug release measurements.
Funding Support:	The rest of Ms. Avellan's support came from a teaching assistantship.

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

Completed Support:
1. Grant "Bioinspired Polymers for Fetal Membrane Pre-Sealing (NIH R01EB022031)" is in no-cost extension through 12/31/2021.

New Support:
1. Grant "Multifunctional tannic acid nanocoatings for bone-anchored implants with reduced infection risk" (University of Oslo, 6/17/2020 – 12/30/2024, Messersmith effort: 0.01 calendar) has been funded. There is no overlap with this grant.
2. Grant "Unraveling the Dark Side of Marine Biotools: Development of Lightweight & Radioprotective Composites" (UC Lab Fees Research Program, Fellowship Award for Katerina Malollari, 03/01/2021-03/30/2023, Messersmith effort: 0.01 calendar) has been funded. There is no overlap with this grant.

What other organizations were involved as partners?

Organization Name:	Lankenau Institute for Medical Research
Location of Organization:	100 East Lancaster Avenue, Wynnewood, PA 19096
Partner's contribution to the project:	
Financial Support:	
In-kind support:	
Facilities:	
Collaboration:	Dr. Heber-Katz is the originating PI on thi grant.
Personnel exchanges:	
Other:	

8. APPENDICES
NA