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TITLE: Enhancing Peripheral Nerve Regeneration with a Novel Drug Delivering Nerve Conduit

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RECIPIENT: University of Utah**, Salt Lake City, UT 84112**

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14. ABSTRACT

This project is directed at improving the rate of nerve regeneration, through the development and testing of a novel nerve conduit. Our technology is a fusion between a biodegradable nerve conduit and a biodegradable drug reservoir. Combat gear for the modern day warrior has greatly improved protection for the head and body, but limbs are still highly exposed to injury. Subsequently, the most frequent combat nerve injuries are in the upper and lower extremities. Patients often suffer from life-long loss or functional disturbances mediated by the injured nerve, which can severely diminish their quality of life. Autologous nerve grafts serve as the state-of-the-art but numerous challenges associated with this approach results in functional benefits to only 40-50% of patients with the additional consequence of nerve-graft donor site morbidity. This project will explore the use of PLGA (poly (lactic-co-glycolic acid) nerve guide conduits that will act as an axon guide for the regenerating nerve. In addition, a drug delivery device will be incorporated with the PLGA conduit in a concentric fashion to deliver nerve growth factor (NGF) and glial cell line-derived neurotrophic factor (GDNF) for at least 30 days to improve rate of nerve regeneration. The use of PLGA or collagen has been tried previously. These devices typically focus on loading the drug in hydrogels or embedding the drug within the conduit. The shortcomings of current devices in terms of burst effect, non-uniform dosage, and uneven drug delivery, necessitates a new approach to deliver drug for nerve regeneration. The currently proposed design is advantageous, as it will allow for controlled drug release that can be tailored made with the ability to vary the concentration, duration, and rate of release of multiple drugs.

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1. INTRODUCTION:

This project is directed at improving the rate of nerve regeneration, through the development and testing of a novel nerve conduit. Our technology is a fusion between a biodegradable nerve conduit and a biodegradable drug reservoir. Combat gear for the modern day warrior has greatly improved protection for the head and body, but limbs are still highly exposed to injury. Subsequently, the most frequent combat nerve injuries are in the upper and lower extremities. Patients often suffer from life-long loss or functional disturbances mediated by the injured nerve, which can severely diminish their quality of life. Autologous nerve grafts serve as the state-of-the-art but numerous challenges associated with this approach results in functional benefits to only 40-50% of patients with the additional consequence of nerve-graft donor site morbidity.
This project will explore the use of PLGA (poly (lactic-co-glycolic acid) nerve guide conduits that will act as an axon guide for the regenerating nerve. In addition, a drug delivery device will be incorporated with the PLGA conduit in a concentric fashion to deliver nerve growth factor (NGF) and glial cell line-derived neurotrophic factor (GDNF) for at least 30 days to improve rate of nerve regeneration. The use of PLGA or collagen has been tried previously. These devices typically focus on loading the drug in hydrogels or embedding the drug within the conduit. The shortcomings of current devices in terms of burst effect, non-uniform dosage, and uneven drug delivery, necessitates a new approach to deliver drug for nerve regeneration. The currently proposed design is advantageous, as it will allow for controlled drug release that can be tailored made with the ability to vary the concentration, duration, and rate of release of multiple drugs.

2. KEYWORDS:

Nerve regeneration, peripheral nerve regeneration, nerve conduits, autograft, drug delivery device, nerve growth factor, glial cell line-derived neutrophic factor, poly (lactic-co-glycolic acid), nerve grafts, axon growth, dorsal root ganglion cells, axon density, motor neuron, sensory neuron

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Specific Aim 1 -- To optimize release kinetics of NGF and GDNF in vitro using our novel drug delivery conduit.

Tasks/Subtasks:

1. Manufacture Devices for use in 15mm nerve gap .....(Gale,)(0-10 months)
    a. Optimize PGLA ratios.....(Gale/Terry)(0-4months)
    b. Optimize nanoporous membrane dimensions.....(Gale)(2-6months)
    c. Optimize reservoir dimensions .....(Gale)(4-8months)
    d. Manufacture and assemble components .....(Gale)(6-10months)

Progress: We have completed these tasks as planned.

Specific Aim 2 -- To evaluate the effectiveness of the conduit-drug delivery device at releasing bioactive concentrations of NGF and GDNF sufficient to enhance axon growth in dorsal root ganglion (DRG) cell culture

Tasks/Subtasks:

1. In Vitro NGF/GNDF release kinetics experiments.....(Gale, Agarwal) (11-18months)
2. ELISA detection of NGF/GDNF .....(Gale, Agarwal) (11-18months)
3. Axonal growth of DRGs .....(Terry, Shea) (11-18months)

Progress: We have started these tasks as planned. Initial release kinetics with NGF analogue, tacrolimus and NGF have been encouraging. We have been able to control the rate of drug delivery for 30 day period at desired dosage. We have optimized the ELISA detection for both NGF and GDNF. We have used NGF and GDNF combination to determine bioactivity of these drugs and optimize dosage concentration by monitoring axonal growth of DRGs. We have completed about 40% of specific Aim 2 tasks and we are meeting the timeline requirements.

Specific Aim 3 -- To evaluate the effectiveness of the conduit-drug delivery device to enhance nerve regeneration across a nerve gap in a mouse sciatic nerve model.

1. IACUC approval, obtain N=160 animals .....(Agarwal, Shea) (19-20 months)
2. Implant Device ½ devices (Mix 30-90 day groups) .....(Agarwal, Shea) (21-25months)
    a. ELISA for NGF/GDNF detection of day 30 animals.....(Gale) (24-29 months)
    b. Walking Track .....(Agarwal, Shea) (21-28months)
    c. Histology.....(Agarwal, Shea) (22-32months) (H&E, immunohistochemistry, retrograde labeling, muscle histology)
    d. Electrophysiology .....(Clark) (24-30months)
3. Implant Device ½ devices (Mix 30-90 day groups) .....(Agarwal, Shea) (26-30months)
    a. ELISA for NGF/GDNF detection of day 30 animals.....(Gale) (29-33 months)
    b. Walking Track .....(Agarwal, Shea) (26-33months)
    c. Histology.....(Agarwal, Shea) (27-34months) (H&E, immunohistochemistry, retrograde labeling, muscle histology)
    d. Electrophysiology .....(Clark) (29-34months)
4. Data Compiling/Analysis and Manuscript Preparation .....(Agarwal, Gale, Shea, Clark, Terry) (30-36months)

Progress: We have obtained the IACUC approval. We have not commenced the animal experiments yet. We have completed 5% of tasks for Specific Aim 3.

What was accomplished under these goals?

Major Activities

Tasks/Subtasks:

Task 1. Manufacture Devices for use in 15mm nerve gap
- a. Optimize PGLA ratios
- b. Optimize nanoporous membrane dimensions
- c. Optimize reservoir dimensions
- d. Manufacture and assemble components

Progress: We have completed these tasks as planned.

Task 2. In Vitro NGF/GNDF release kinetics experiments
Task 3. ELISA detection of NGF/GDNF
Task 4. Axonal growth of DRGs
Progress: We have completed about 40% of specific Aim 2 tasks and we are meeting the timeline requirements.

Task 5. IACUC approval, obtain N=160
Task 6. Implant Device ½ devices (Mix 30-90 day groups)
- a. ELISA for NGF/GDNF detection of day 30 animals
- b. Walking Track
- c. Histology (H&E, immunohistochemistry, retrograde labeling, muscle histology)
- d. Electrophysiology

Task 7. Implant Device ½ devices (Mix 30-90 day groups)
- a. ELISA for NGF/GDNF detection of day 30 animals
- b. Walking Track
- c. Histology (H&E, immunohistochemistry, retrograde labeling, muscle histology)
- d. Electrophysiology

Task 8. Data Compiling/Analysis and Manuscript Preparation
Progress: We have obtained the IACUC approval. We have not commenced the animal experiments yet. We have completed 5% of tasks for Specific Aim 3.

Specific Objectives

Specific Aim 1 -- To optimize release kinetics of NGF and GDNF in vitro using our novel drug delivery conduit.
Specific Aim 2 -- To evaluate the effectiveness of the conduit-drug delivery device at releasing bioactive concentrations of NGF and GDNF sufficient to enhance axon growth in dorsal root ganglion (DRG) cell culture
Specific Aim 3 -- To evaluate the effectiveness of the conduit-drug delivery device to enhance nerve regeneration across a nerve gap in a mouse sciatic nerve model.

Significant Results

Device Fabrication.

A bridging technique with the aid of NGF was proposed to repair peripheral nerve gaps. We have fabricated several generations of the device iterated towards a completely bioresorbable device.

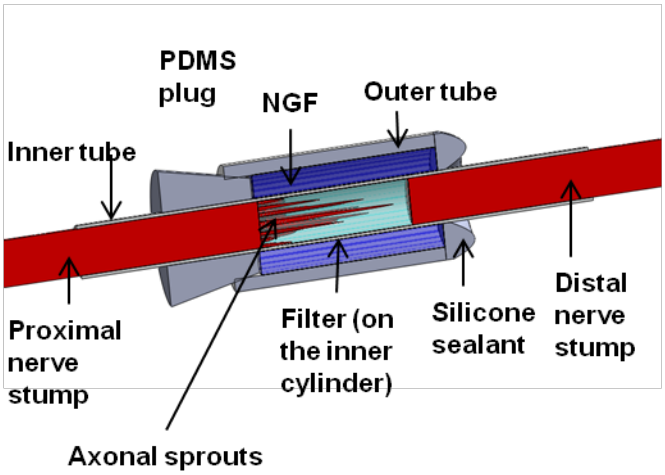


Figure 1 Sketch of a PLGA nerve conduit. Drug (NGF) loaded in the orifice between the outer and inner tubes will diffuse through the filter and enter the orifice of the inner tube, contacting nerve stumps and stimulating axon growth on the proximal nerve stump. The inner tube can fix the two nerve stumps and guide the new-grown axon to meet the distal nerve stump. Silicone sealant and a PDMS plug are used to seal and fix the two tubes.

First generation device: Due to its relatively short half life compared to 50/50 PLGA, PLGA in 75 to 25 poly-lactic-acid (PLA) to poly-co-glycolic-acid (PGA) copolymer ratio was used to form the outer and inner tubes of the PLGA nerve conduit. A polyether sulfone (PES) filter membrane, a polydimethyl siloxane (PDMS) plug and silicone sealant (RTV silicone, Dow Corning Inc) were also added to the nerve conduit. Figure 2 illustrates the nerve conduit in which the drug (NGF) stored in the orifice between two concentric tubes can release into the orifice of the inner tube through the filter, and thus contact to the proximal nerve stump.

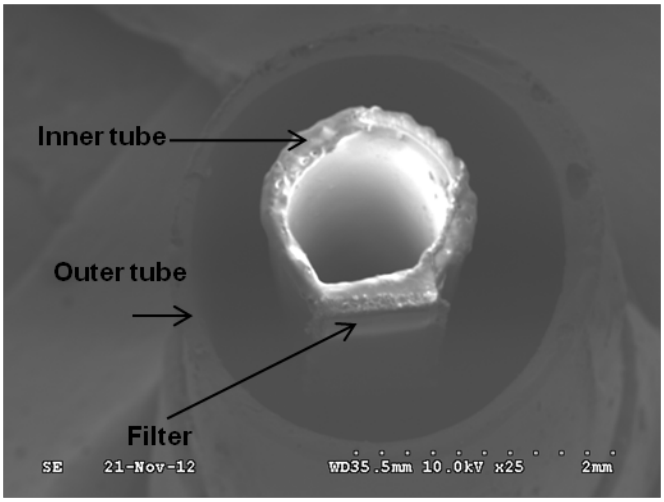


Figure 2 Cross-sectional view of the PLGA nerve conduit. The filter is attached on a window on the inner tube to allow the drug (not shown) stored between the inner tube and the outer tube to release into the orifice of the inner tube and promote local axonal outgrowth on the proximal nerve stumps.

Second generation device: All PLGA Conduit

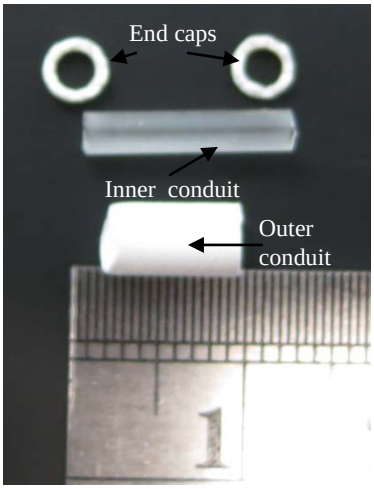


Figure 4 Photograph of the second generation device components (completely bioresorbable—all PLGA).

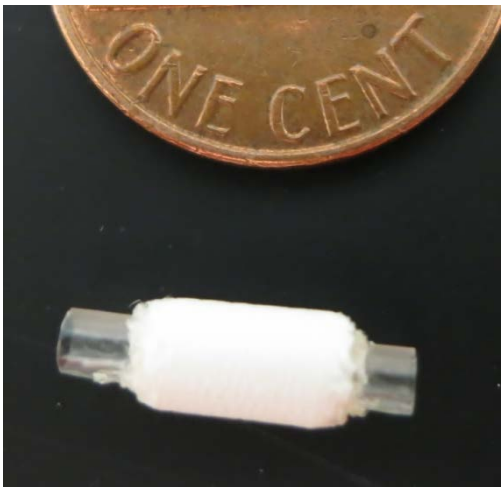


Figure 3 Photograph of an all PLGA device with laser machined holes for drug diffusion area

The bioresorbable guidance conduits were produced using 75/25 poly-lactic-glycolic-acid (PLGA; 7525 DLG 7E, Evonik). The PLGA was dissolved in acetone and ethanol and conduits were then formed and emulsified in water. 15µm diffusion holes were drilled into the inner conduit by pulsing a laser cutter. Final assembly of mold-formed dual conduits and end caps was done using a solvent bonding process, resulting in two ~15µl drug reservoirs.

(a) (b) (c)

Figure 3. (a) Phtograph of the nerve conduit parts: end caps, inner conduit and outer conduit and (b) Photograph of the complete single reservoir conduit, (c) Photograph of the conduit with food coloring to demonstrate device integrity.

(a)

Figure 4. (a) Photograph of a complete dual reservoir chamber with dyes of different colors in two chambers.

Initial NGF release study.

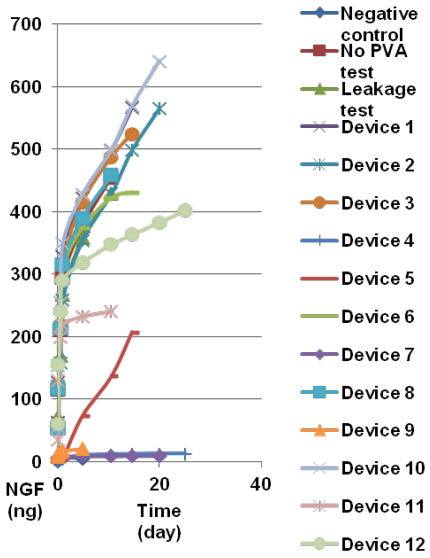


Figure 5 Cumulative NGF amount released into the receiver chamber. In each collection, all the media in the receiver chamber was replaced with fresh media. Thus, the sum of NGF concentration detected in each collection was shown in this figure to present the cumulative amount of NGF released from the PLGA device at each time point.

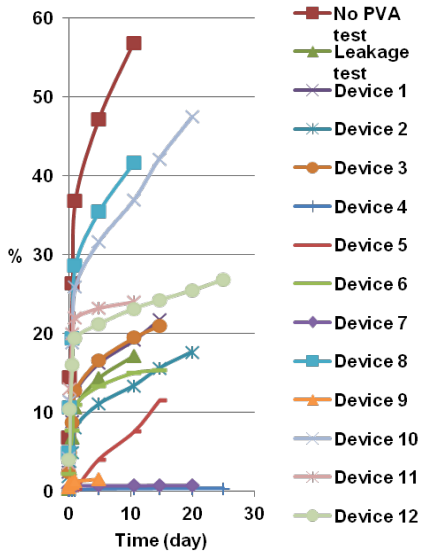


Figure 6 Cumulative percentage of NGF released into the receiver chamber in the 25 day diffusion test.

Devices were triplicate, and the combination of NGF in either 0.1mg/mL or 0.05mg/mL and PVA in either 25mg/mL or 12.5mg/mL will be filled into each PLGA nerve conduit (device). A no PVA test filled with only 0.05mg/mL NGF without PVA was added in order to verify the situation when the absence of PVA will result in faster NGF release, Because the role of PVA is to block the PES filter membrane partially and slow down (control) the NGF release so that the NGF can be released constantly in the entire 25-day period. A leakage test was also added in order to verify the leakage of the device. A special PLGA conduit without either the window on

the inner tube or the PES filter membrane was built and used in this test, and near zero NGF release is expected. 40μL 0.1mg/mL NGF with 25mg/mL PVA solution in 4mL receiver chamber media in triplicate copies served as the Positive Control. Negative control involved only 4mL receiver chamber media in triplicate copies. For both devices and controls, Dulbecco's modified Eagle's medium (DMEM, SH3026101, Thermo Scientific) with 10% fetal bovine serum (FBS, SH3091003, Thermo Scientific) was used as the medium for the receiver chamber. This solution was used so that the media from the receiver chamber could be directly used to treat neural cells in order to verify the bioactivity of NGF in the next experiment.

50mm polypropylene Petri dishes were used as the chamber for this 25-day diffusion test. Each PLGA nerve conduit was filled with the desired drug and sealed using the PDMS plug end with silicone sealant. The device was dried for 1 hour and then mounted individually onto the bottom of the Petri dish with silicone sealant. An additional 30 minutes was added to ensure the silicone sealant was dry before the application of the media to the receiver chamber. After applying 3mL DMEM with 10% FBS into each dish, the Petri dishes were transferred into a 37ºC incubator. Devices and controls would be collected after 1, 4, 14, 25, 117, 254, 351, 480 and 600 hours. During device collection, the entire media of the receiver chamber will be replaced with fresh DMEM with 10% FBS (sink method) so that the information of NGF released into the receiver chamber in each period could be obtained. An NGF ELISA kit (ab100757, ABCam) was used to analyze the NGF concentration at each time point for both devices and controls.

NGF concentration for both devices and controls in the 25-day diffusion test was obtained from ELISA, and data were processed through Microsoft Excel. Contaminations in the diffusion chamber (the Petri dish) were found in some devices and controls starting from the 10^{th}-day collection, and the ones with contamination were discarded without collection or measurement.

Figure 5 shows the cumulative NGF amount released from the PLGA device into the receiver chamber (Petri dish) at each time point. Since all the media in the receiver chamber was replaced with fresh media during each collection (sink method), concentration data detected from the NGF ELISA were converted into NGF in weight (ng) and the results were added up at each time point. Negative control shows a 6ng cumulative release among the first five days, and positive control shows a 103ng release. The leakage test shows a cumulative 429ng NGF release during the 10 day period; the no PVA test shows a cumulative 455ng NGF release in the same 10 day period. Among the twelve devices, only three devices (Devices 4, 7 and 9) show a relatively low release with a final cumulative NGF release of 13, 9 and 21ng, respectively. Devices were prepared in triplicate and therefore Devices 1-3, 4-6, 7-9 and 10-12 share the same dosage of PVA and NGF, though the volume is different, as shown in Table 1. Most of the devices show a two-step release, during which a burst release was observed between the start and the 1^{st} day (25 hr) collection. After the 1^{st}-day collection, a slower release was obtained for most of the devices. For Devices 1, 2, 3, 5, 6, 8, 10, 11 and 12, the average NGF release for the first day, between the 1^{st}- and 10^{th}-day, between the 1^{st}-and 15^{th}-day, and between the 1^{st}-and 20^{th}-day, if available, are 256ng/day, 13ng/day, 14ng/day and 12ng/day, respectively.

Figure 6 shows the cumulative percentage of NGF released into the receiver chamber at each time point. The purpose for showing these data is to inspect how much NGF released during the 30-day period in order to know if a longer drug release period can be achieved in the future when required. The slopes for the data in this figure are different than the ones in Figure 5 because

different volume and concentration of NGF were filled into the devices and controls. There is no data for Negative Control in this figure since no NGF was added in this control. For no PVA test, without the presence of PVA, 56.9% of NGF was released into the receiver chamber in the 10-day period. Only 17.1% of NGF was released from the Leakage Test's PLGA device. As for the device designed to release NGF among the 25-day period, Device 10 has the highest NGF release as 47.5% is released into the receiver chamber in the 20-day period. Devices 4, 7 and 9 have the lowest NGF release in the diffusion test at 0.4, 0.8 and 1.5 percent, respectively. Device 5 has a zero-order release and release 11.5% NGF during the 15-day period. For all the other devices, a two-step release curve was also obtained, and the average release for the first day, between the 1st-and the 10th-day, between the 1st-and the 15th-day and between the 1st-and the 20th-day is 16.85, 0.69, 0.62 and 0.65 percent, respectively.

Diffusion Modelling

Fick's Diffusion

1st Law: $J = -D\frac{\partial \Phi}{\partial x}$

- Assumes steady state

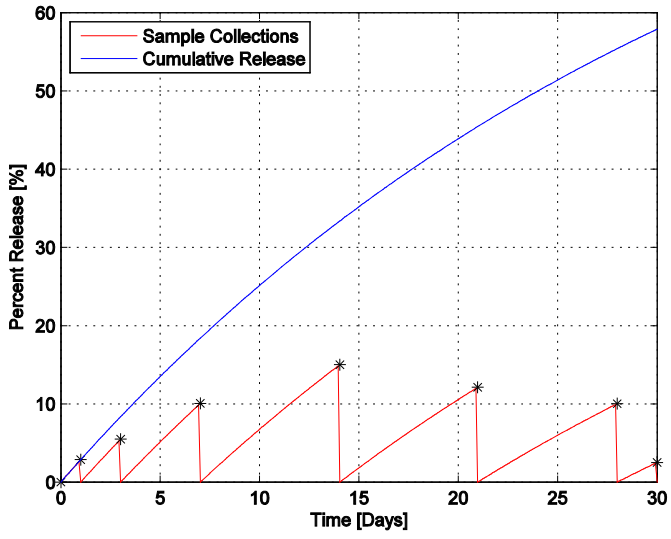
2nd Law: $\frac{\partial \Phi}{\partial t} = D\frac{\partial^2 \Phi}{\partial x^2}$

NGF/Dextran $D_{NGF} = 1.26*10^{-6}$ cm^2/sec

*Dextran has equivalent diffusion coefficient as NGF, used as simulated drug

Targets

- ~60% release in $\tau = 30$ days
- Drug diffusion can be independently tuned by adjusting diffusion hole size or initial drug concentration
- Receiver chamber flushing taken into account



DRG Studies

In the experiments, the harvested DRGs were treated with increasing concentrations of NGF, GDNF and both NGF/GDNF. The dilutions were made with the DRG culture medium.

Quantification of neurite outgrowth: axonal length and density

The area of the ganglion body (ADRG) and the total area of the DRG with the growing axons (Atot) were measured using ImageJ 1.31v software (National Institutes of Health, Bethesda, USA). The average axonal length (lavg) was calculated by: lavg = (Atot/π)1/2 − (ADRG/π)1/2. The axonal density was calculated from the area occupied by the axons (Aaxon), which was determined using the threshold function available in ImageJ: Aaxon/(Atot − ADRG).

Brightfield/darkfield images of DRGs were taken after 3 days of incubation, and the average axonal length and density was measured as described above.

Cultures of dissociated DRG neurons

DRGs were harvested from chicken embryos. 1 DRG/well was plated in 24-well plates coated with laminin (1 µg/ml). Finally, different doses of NGF, GDNF and NGF/GDNF were added, and the cultures maintained for 3 days in an incubator.

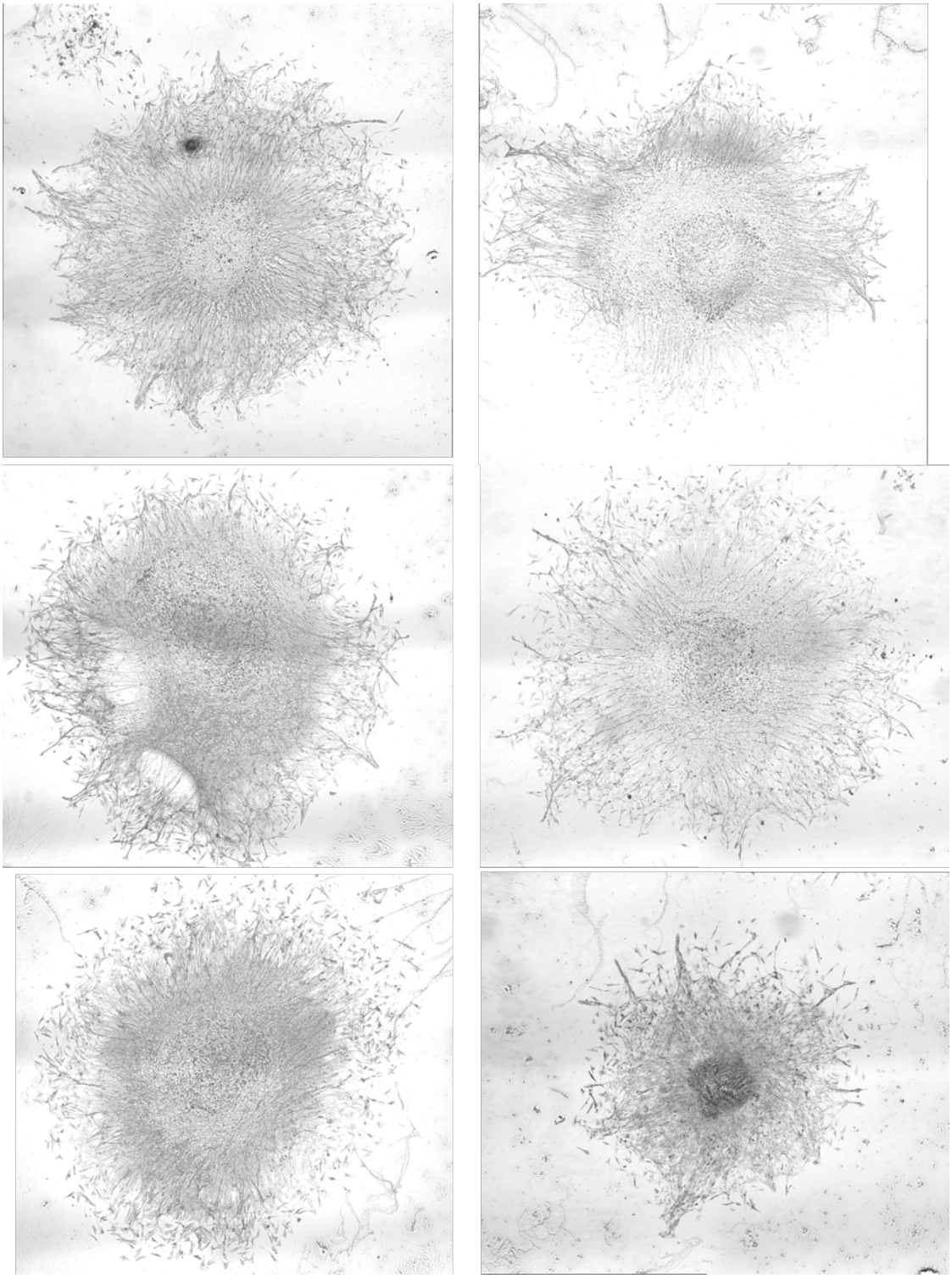
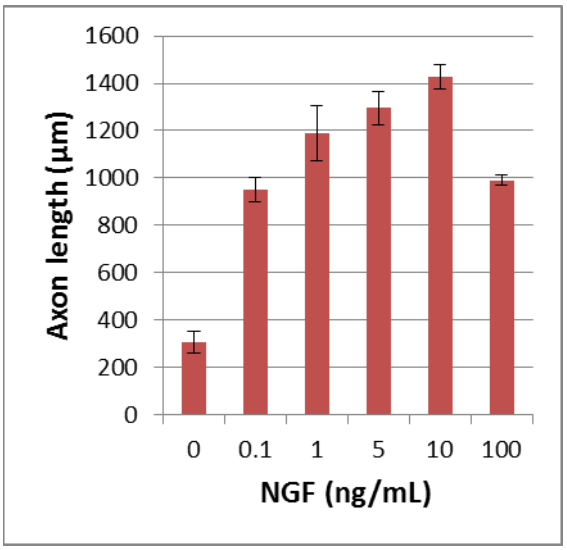
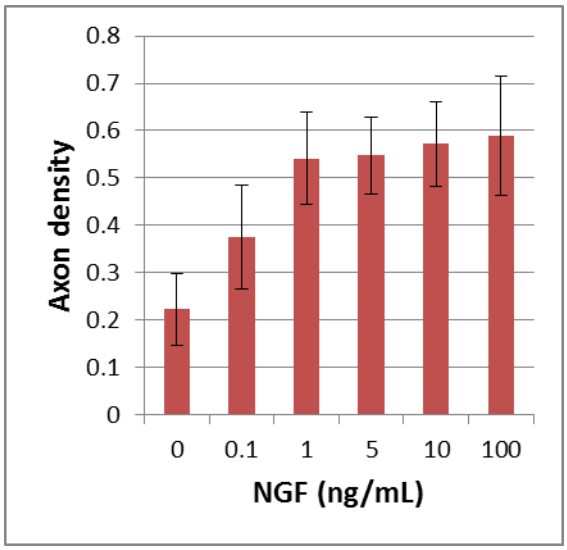


Fig. Starting from top (L to R) 1 ng/mL NGF 0.1 ng/mL GDNF, 100 ng/mL NGF 100 ng/mL GDNF, 0.1 ng/mL NGF 0.1 ng/mL GDNF, 0.1 ng/mL NGF 0 ng/mL GDNF, 0 ng/mL NGF 1 ng/mL GDNF, 10 ng/mL NGF 1 ng/mL GDNF

NGF dosage curve for DRGs

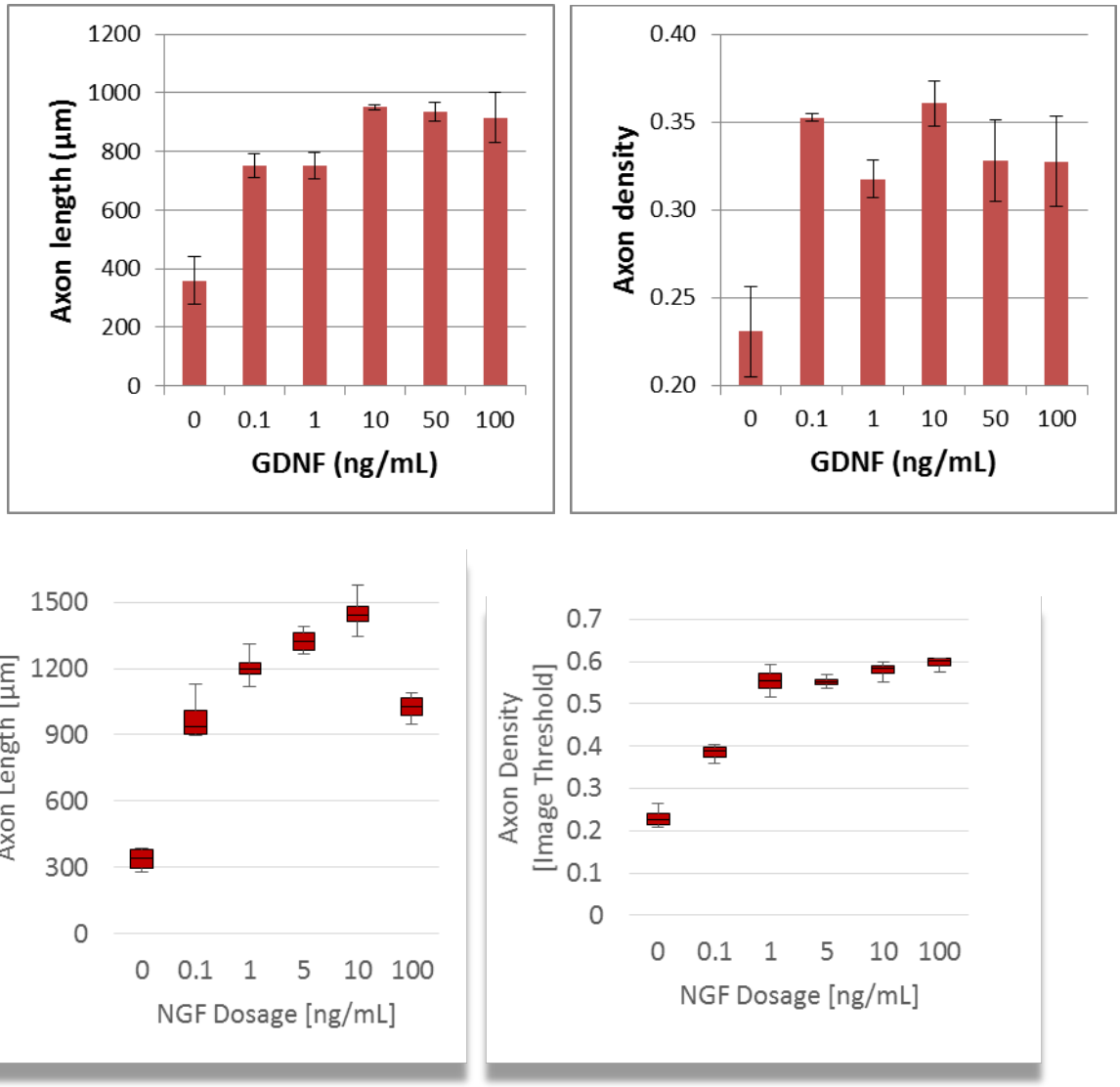
	Axon length µm	Axon density
NGF (ng/mL)	Avg (n=4)	Avg (n=4)
0	304.35	0.22
0.1	950.20	0.37
1	1188.70	0.54
5	1295.84	0.55
10	1427.79	0.57
100	990.59	0.59





GDNF Dosage curve for DRGS

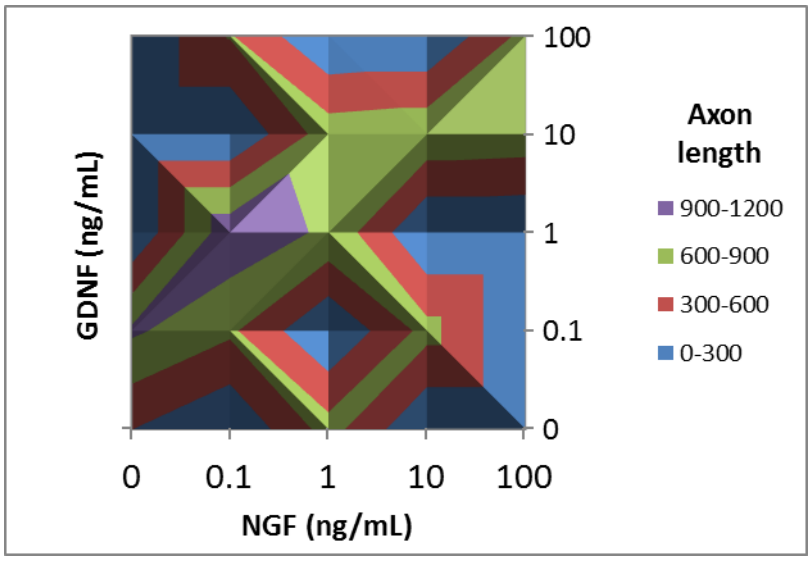
	Axon length (µm)	Axon density
GDNF (ng/mL)	Avg (n=4)	Avg (n=4)
0	358.19	0.23
0.1	749.77	0.35
1	751.96	0.32
10	950.85	0.36
50	936.26	0.33
100	914.58	0.33



NGF/GDNF combined dosage curve for DRGs

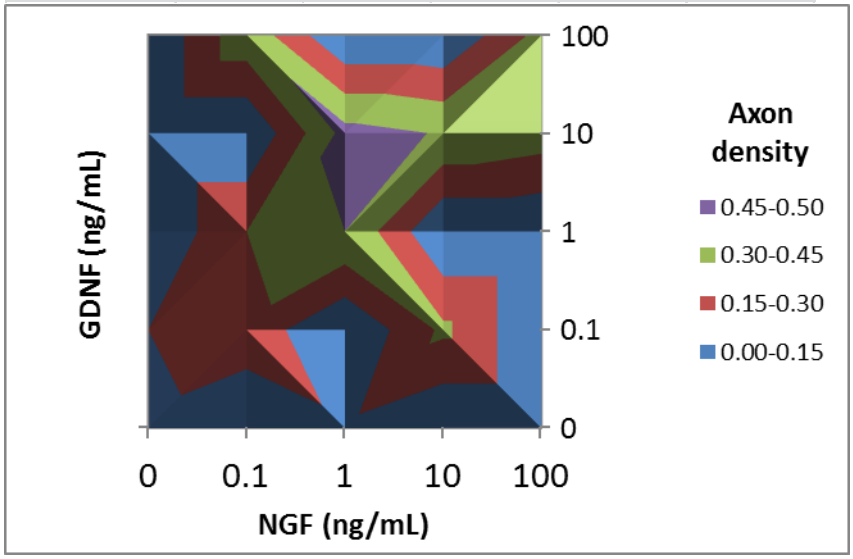
Axon length

	GDNF (ng/mL)				
NGF (ng/mL)	0	0.1	1	10	100
0	304		724		
0.1	950	656		703	
1		1110	848		
10			759	819	784
100		622			689



Axon density

	GDNF (ng/mL)				
NGF (ng/mL)	0	0.1	1	10	100
0	0.10		0.12		
0.1	0.15	0.25		0.33	
1		0.30	0.45		
10			0.57	0.44	0.38
100		0.41			0.35



Discussion

Treatment of the DRG-explants with GDNF or NGF induced readily visible axonal outgrowth as compared with control explants. GDNF promoted primarily axonal elongation with little axonal branching, whereas NGF induced extensive axonal branching with axonal elongation

Further, the axonal elongation was dose-dependent with both GDNF (within range of 0.1–10 ng/ml) and NGF (within range of 0.1–10 ng/ml). At 100 ng/ml of NGF or GDNF, axonal elongation was compromised which suggested an optimal growth factor concentration of ~10 ng/ml. Finally, the axonal density of the NGF-treated DRG was generally about 20% above that observed with the GDNF-treated explants.
Axonal density, a measure of axonal branching, was strongly promoted by NGF even at the very low concentration of 0.1 ng/ml. GDNF induced significant axonal branching only at the concentration of 10 ng/ml.

Considering the individual growth effects of GDNF and NGF on the axonal outgrowth, we used the two growth factors in combination. Combined dosage of GDNF and NGF produced significant enhancement of axonal outgrowth as compared with the single factor treatments. The plots suggest a strong effect of increasing NGF concentration (0.1, 1, and 10 ng/ml). The average axonal length at optimal GDNF/NGF concentrations (1 ng/ml NGF combined with 1 or 0.1 ng/ml GDNF) was in the order of 0.9–1 mm, which compares to the 0.8–0.9 mm axonal elongation observed at 1–10 ng/ml GDNF single treatment. Altogether, the results indicated a strong interaction of both growth factors. This interaction seemed to be synergistic for axonal growth at all GDNF and NGF concentrations. Axon density measurements seemed more NGF dose-dependent rather than GDNF dose-dependent. Axonal density augmentation was more of a competitive interaction at lower GDNF concentrations and synergistic at higher GDNF values >1 ng/mL

Dextran release

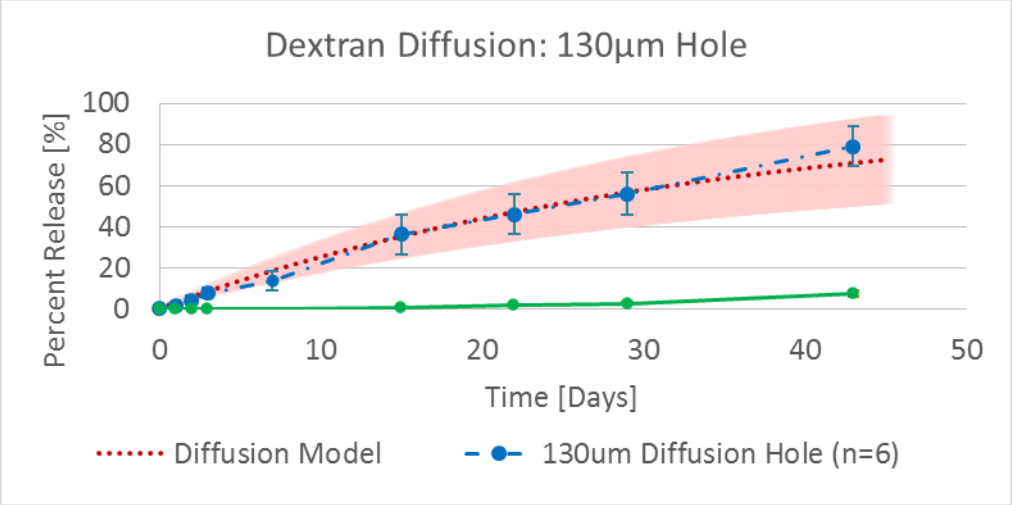


Figure 9. Release data for NGF with single 130 um hole. NGF analogue, Dextran, was used for drug delivery. The device is an all PLGA with inner conduit and PLGA drug reservoir. A CO2 laser is used to create diffusion hole in PLGA conduit. No filter membrane is used for this device thus simplifying manufacturing.

Setup Parameters

- Single reservoir devices (non-sterile)
- Target release: ~60% diffusion over 30 days

- 1x 130µm hole per reservoir
- Loaded drug: Texas Red Dextran
- ~20µL @10mg/ml (target: fluorescence range)
- Receiver chamber: 3mL PBS (saline)
- Tested @21°C

Results

- Drug diffusion falls within target region
- Tests will continue to run for 120 days

Conclusions

- Repeatable manufacturing and diffusion

NGF release

- Drugs are loaded into device reservoirs
- Device is placed into receiver chamber
- Receiver chamber is filled with known volume of collection fluid
- Receiver chamber medium is collected on determined time intervals
- Following sample collection, receiver chamber is flushed and new fluid is added
- Sample fluorescence is read using a plate reader and data is fitted to its standard curve
- ELISA kits used for NGF samples
- Data is processed and cumulative release is determined

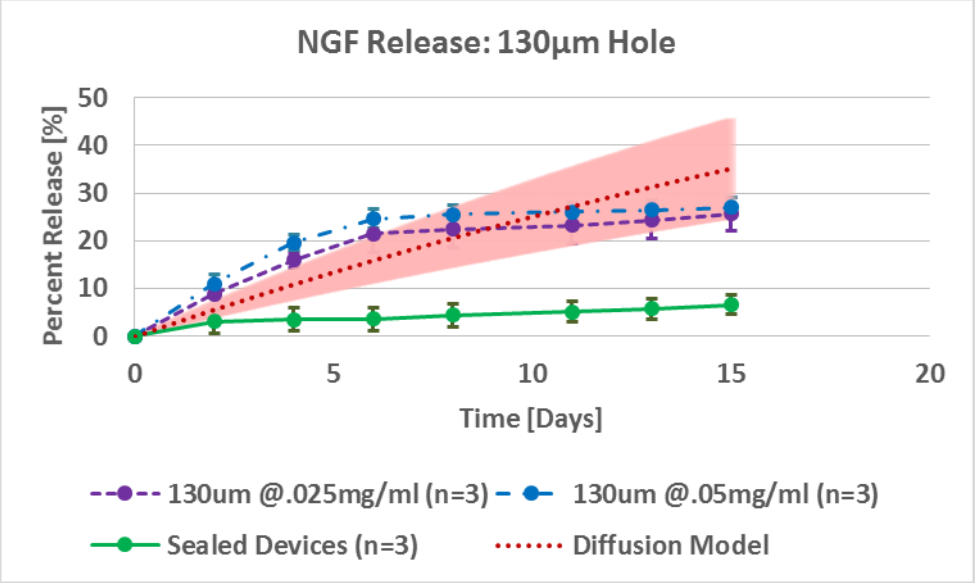


Figure 7 Release data for NGF with single 130 um hole. The device is an all PLGA with inner conduit and PLGA drug reservoir. A CO2 laser is used to create diffusion hole in PLGA conduit. No filter membrane is used for this device thus simplifying manufacturing.

Experimental Parameters

- Single reservoir devices (sterile)
- Target release: ~60% diffusion over 30 days, 2-10ng/ml/day
  - 1x 130µm hole per reservoir

- Loaded drug: NGF
    - ~20µL @.05mg/ml (target: 14ng/ml/day)
    - ~20µL @.05mg/ml (target: 7ng/ml/day)
- Receiver chamber: 3mL media/FBS matrix
    - Changed to PBS after 6 days to minimize contamination effects
- Tested @37°C

Results

- Tests resulted in contamination after 2 days
    - PLGA and NGF have been shown to break down enzymatically and under adverse pH levels
- Diffusion dropped dramatically after 6 days

Figure 8 shows that the diffusion rate is independent of loaded concentration. Additionally, the dosage values can be adjusted linearly.

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

1. Completion of PhD research project for Keng-Min Lin.
2. Continuation of PhD research project for Pratima Labroo
3. Continuation of MS research project for Scott Ho.
4. Undergraduate research project for Megan and Renee

How were the results disseminated to communities of interest?

Nothing to Report

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

The Next report will be the quarter 1 report for Year 2 of this project. We expect to report outcomes of further testing bioactivity of NGF & GDNF with DRG cells and verification of axonal outgrowth in DRG cells with eluate of the device

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

We will use reduced factorial design of experiments to optimize freshly prepared and 30 day incubated NGF and GDNF combinations based on DRG cell axonal growth. The optimized NGF/GDNF combination will be used to eluate through the drug delivery device and bioactivity of the same will be verified with DRG cells.

4. **IMPACT:** Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

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

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

As part of this work, we have developed a new mathematical model that can be used by researchers to predict, reservoir volume, drug amount, drug concentration, and diffusion hole size. This model will help researchers to avoid costly and time intensive in-vitro trials.

We have developed fabrication and sterilization protocols for completely biodegradable device with dual drug reservoirs and tested the efficacy of the device using in-vitro and DRG studies. This data will help researchers/industry to further develop drug delivery efforts in other areas as well.

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

Nothing to report

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

Nothing to report

5. **CHANGES/PROBLEMS:** The Project Director/Principal Investigator (PD/PI) is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer 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:

Changes in approach and reasons for change
Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

Nothing to Report.

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

None.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Significant changes in use or care of human subjects

No Change.

Significant changes in use or care of vertebrate animals.

No Change.

Significant changes in use of biohazards and/or select agents

No Change.

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.

  1. Keng-Min Lin, Bruce K. Gale, Himanshu Sant, Jill Shea, Scott Ho and Jay Agarwal. Drug-delivery nerve conduits for peripheral nerve regeneration, Journal of Micromechanics and Microengineering, in preparation, acknowledgement of federal support (yes)
  2. Keng-Min Lin, Bruce K. Gale, Himanshu Sant, Srinivas Chennamaneni, Michael Burr and Jay Agarwal. PDMS drug delivery devices: potential application in nerve regeneration, Biomedical Microdevices, in preparation, acknowledgement of federal support (yes)

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

Keng-Min Lin, IMPLANTABLE DEVICES FOR SENSING AND DRUG DELIVERY IN OPHTHALMOLOGY AND RECONSTRUCTIVE SURGERY, Ph. D. Dissertation, Department of Mechanical Engineering, University of Utah, May 2014, acknowledgement of federal support (yes)

Other publications, conference papers, and presentations.

Scott Ho, Pratima Labroo, Keng-Min Lin, Himanshu Sant, Jill Shea, Jay Agarwal, Bruce Gale, Bioresorbable Multi-Drug Delivery Conduit to Promote Peripheral Nerve Regeneration, in Proceedings of 2014 BMES Annual Meeting, San Antonio, Texas, October 22-25, 2014.

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

http://www.mems.utah.edu/publications/
This website lists the publications and research originating from Co-PI Dr. Gale's lab.

- **Technologies or techniques**

Fabrication of biodegradable drug delivery prototypes using PLGA. We will publish journal articles to share the device fabrication techniques.

- **Inventions, patent applications, and/or licenses**
Identify inventions, patent applications with date, and/or licenses that have resulted from the research. State whether an application is provisional or non-provisional and indicate the application number. 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.

1. SANT HIMANSHU JAYANT, GALE BRUCE KENT, AGARWAL JAYANT P, LIN KENG-MIN, METHODS AND DEVICES FOR CONNECTING NERVES, Last status change:2013-05-10/ Fill date:2012-10-16, WO 2013066619

- **Other Products**
  1. Mathematical model based on Fick's diffusion law
  2. Fabrication of dual chamber combined PLGA nerve guide and drug delivery device prototypes
  3. Use of laser to create diffusion hole

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."

Personnel	Role	Percent Effort	Months
Jay Agarwal	PD/PI	8.33	1.0
Overall management of the project, guidance to students, weekly meetings and report preparation.			
Bruce Gale	Co-I	7.83	1.0
Device manufacturing, weekly meetings			
Jill Shea	Co-I	16.5	2.0
IRB approvals, DRG studies, ELISA, histology preparation, animal studies prep., weekly meetings.			
Himanshu Sant	Co-I	21.16	2.5
Device manufacturing and validation, mathematical model, weekly meetings and report preparation.			
Christi Terry	Co-I	13.5	1.5
DRG studies, ELISA optimization			
Gregory Clark	Co-I	5.25	1.0
Electrophysiology protocol preparation			
Keng-Min Lin	Student	50.00	6.0
Device manufacturing, mathematical model and ELISA			
Scott Ho	Student	50.00	6.0
PLGA dual chamber prototype fabrication, mathematical model, weekly meetings.			
Pratima Labroo	Student	25.00	4.0
DRG studies, ELISA, animal studies training, and weekly meetings.			

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

Nothing to Report

What other organizations were involved as partners?

None.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: For collaborative awards, independent reports are required from BOTH the Initiating 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.

None.

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.

Bioresorbable Multi-Drug Delivery Conduit to Promote Peripheral Nerve Regeneration

Scott Ho[1], Keng-Min Lin[1], Dr. Himanshu Sant[1], Dr. Jill Shea[2], Dr. Jay Agarwal[2], Dr. Bruce Gale[1]
Department of Mechanical Engineering, University of Utah[1]
Department of Surgery, University of Utah[2]

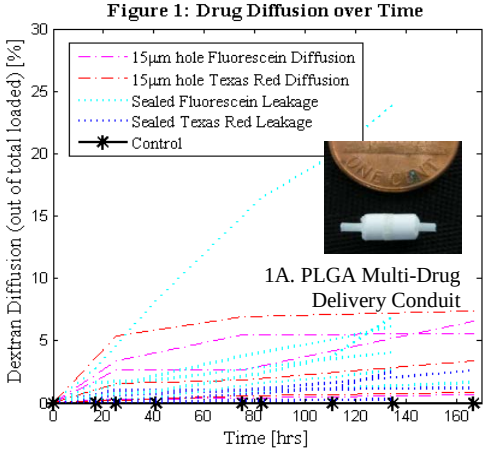
Introduction: Peripheral nerve lesions caused by trauma often require the removal of the injured segment of nerve and subsequent repair by surgery. Synthetic nerve guidance conduits currently are commercially available but they have proven ineffective in promoting sufficient axonal growth. There are major benefits in providing a guidance conduit that can independently deliver multiple localized drugs to the injury site. A bulk diffusion delivery device will provide flexibility in easily alternating drugs as well as precision in using traditional fluid mechanics to control delivery rather than complex polymer degradation. Diffusion kinetics tests were performed to show that this device is capable of releasing drug at a consistent rate over a 30-day period.

Materials and Methods: The bioresorbable guidance conduits were produced using 75/25 poly-lactic-glycolic-acid (PLGA; 7525 DLG 7E, Evonik). The PLGA was dissolved in acetone and ethanol and conduits were then formed and emulsified in water. 15µm diffusion holes were drilled into the inner conduit by pulsing a laser cutter. Final assembly of mold-formed dual conduits and end caps was done using a solvent bonding process, resulting in two ~15µL drug reservoirs.

Two tests have been performed: an initial sealing test and a pilot diffusion kinetics test. Two types of Dextran were used to replicate drug kinetics to test the conduits: Fluorescein (D1821, Molecular Probes; Ex. 494 Em. 521) and Texas Red (D1863, Molecular Probes; Ex. 595 Em. 615). These simulated drugs were loaded into independent conduit reservoirs and then placed into a receiver chamber filled with phosphate buffered saline (PBS). A series of sample collections were taken from the receiver chamber over specified time intervals and the chamber was flushed and filled with fresh PBS each time. Florescence readings were taken using a microplate reader and the data was analyzed using MATLAB software to determine drug release kinetics.

Results and Discussion: Figure 1 shows the results for initial release kinetics testing (device shown in 1A). First, this test was effective in showing independent release of multiple drugs. The results of the sealed leakage tests (n=5) validate sealing techniques for the drug-release reservoirs. Over a 5-day period all but one of the devices maintained a cumulative leakage under 10% of total drug release, with over half of the devices maintaining a cumulative leakage under 3%.

Figure 1: Drug Diffusion over Time



1A. PLGA Multi-Drug Delivery Conduit

The diffusion tests (n=7) indicate that 15µm holes allow for a sustainable drug release for much longer than 30 days. The original target diffusion was ~7% diffusion over a 30-day period in order to maintain ~0^{th} order diffusion kinetics. However, these pilot tests show that some inconsistencies in manufacturing or compounded diffusion error can overwhelm the intended diffusion. In order to optimize drug release, a higher diffusion target (τ=30 days, ~63% release) will be attempted to overcome minor unexpected errors while still maintaining a relatively constant drug release.

Conclusions: Results from initial leakage tests indicate successful manufacturing techniques in sealing the devices. Current diffusion through a 15µm hole shows that this device is currently capable of sustaining drug release for 3+ months. Larger holes and/or an array of holes will be tested to optimize drug release over 30 days. Inconsistencies in device quality and diffusion precision will continue to be improved and following sufficient release kinetics tests, in-vitro testing using known effective growth factors will be performed to explore the biological efficacy of the device.

Acknowledgements: This project has been funded by the Department of Defense Congressionally Directed Medical Research Programs Discovery Award and Idea Development Award. Additional thanks to Jeremy Riley for aid in manufacturing.