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TITLE: **Large Extremity Peripheral Nerve Repair in Nonhuman Primate Models**

PRINCIPAL INVESTIGATOR: **Robert W. Redmond Ph.D.**

CONTRACTING ORGANIZATION: **Massachusetts General Hospital
Boston, MA 02114**

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1. Introduction.

The goal of the research performed in this project is to develop a new technology for repair of peripheral nerve injuries involving significant neural deficit with improved functional outcomes for the wounded warrior. The research addresses drawbacks of current methods of suture attachment of nerve grafts and involves development of a sutureless fixation method to place the nerve graft and a biocompatible nerve wrap to seal the endoneurial environment for regeneration. Reduction in needle trauma, reduced inflammation and scarring and sealing the endoneurial environment should all contribute to improved clinical outcomes. Prior experiments in small animal models (rodents and rabbits) provided positive results in terms of nerve regeneration and the current award progresses to non-human primate models where both functional recovery testing and electrophysiology can be performed. Of particular importance is the use of acellular nerve allografts (ANA) that could be of major impact in combination with photosealing to improve outcomes in wounded warfighters that lack donor autologous nerve due to major traumatic injury.

2. Keywords: Nerve injury, nerve gap, nerve wrap, acellular nerve allograft, Avance, PTB, photosealing, Rose Bengal, amnion, nerve conduit, crosslinking, allograft, photochemistry.

3. Accomplishments:

What were the major goals of the project?

The overall goal of this JWMPR proposal “Large Extremity Peripheral Nerve Repair in Non-Human Primate Models” is to employ a relevant large animal model of peripheral nerve injury involving large segmental deficit in the upper limbs that recapitulates human anatomy and is capable of objective functional outcome testing that is not possible in other large animal models, such as sheep and swine. With this model we will determine whether our improved method of restoring continuity to peripheral nerves by photosealing a commercial acellular nerve allograft across the deficit can produce outcomes equivalent to standard of care autologous grafting for wounded warfighters that do not have sufficient autologous donor nerve due to extensive combat trauma. Improving functional recovery for wounded warfighters in this manner would have a major impact on quality of life and well-being of such wounded warfighters.

Milestones for this award are listed below, along with percentage completion to date (in bold) where appropriate.

- A. Regulatory approval (MGH IACUC and ACURO) of injury and repair models in Rhesus monkeys (Months 1-4). **100% complete**
- B. Purchase and receipt of laboratory supplies. (Months 1-2) **50% complete**
- C. Harvest of human amniotic membrane (HAM) from placenta obtained from MGH under Discarded Tissue Protocol (Months 1-3). **100% complete**
- D. Crosslinking of HAM with EDC/NHS to make xHAM (Months 2-4). **100% complete**
- E. First meeting of MGH team with surgical advisors at MGH to discuss progress and suggest improvements to the research approach (Month 6). **100% complete**
- F. Purchase and acclimatization of twenty Macaque Monkeys (Months 4-8 at 4 per month). **100% complete.**
- G. Training of monkeys in behavioral task used to evaluate for functional recovery after repair (Months 4-9). **75% complete**
- H. Survival surgeries for peripheral nerve defect and repair in Rhesus monkey model (Months 5-10). **60% complete**
- I. Second meeting of MGH team with military surgeons advisors at MGH to discuss progress and suggest improvements to the research approach (Month 12).
- J. Serial monthly behavioral testing for recovery of function in monkeys. (Months 5-22). **30%**

complete

- K. Serial electrophysiology measurements in monkeys (Months 5-22). **10% complete**
- L. Third meeting of MGH team with military clinical advisors at MGH to discuss progress and suggest improvements to the research approach (Month 18).
- M. Euthanasia of Monkeys at one-year post-operative time points (Months 17-22).
- N. Harvest of nerve repair complex at euthanasia and preparation of samples for histology and histomorphometry (Months 17-22).
- O. Harvest of innervated muscle at euthanasia and determination of retention of muscle mass with respect to unoperated contralateral limb. (Months 17-22).
- P. Analysis of histomorphometric data from nerve cross-sections. (Months 22-24).
- Q. Final meeting of MGH team with military surgeons advisors at MGH to discuss results and conclusions from the entire project and plan for immediate human trial, if successful. (Month 24).
- R. Preparation of report and manuscript based on outcomes

What was accomplished under these goals?

Task A - Regulatory approval (MGH IACUC and ACURO) of injury and repair models in Rhesus monkeys.

The institutional animal use protocol for this study (MGH #2016N000627) was submitted at the end of 2016 (see timeline in subsequent section) and approved by the MGH IACUC in March, 2017. A subsequent submission to ACURO in late March led to approval of the protocol by ACURO in June 2017. In planning the studies in full detail it was required to amend the protocol to operate on the right, rather than left arm due to constraints of the animal housing and behavioral testing, to perform a pre-operative EMG to obtain baseline nerve conduction results and for additional study staff. The amended protocol was submitted to MGH IACUC in October and approved in November. The amended protocol gained ACURO approval in early December 2017.

Task B - Purchase and receipt of laboratory supplies.

All supplies necessary for preparation of biocompatible wrap, for electrophysiology, for videography and analysis of behavioral testing and for animal surgeries, were purchased and received.

Task C - Harvest of human amniotic membrane (HAM) from placenta obtained from MGH under Discarded Tissue Protocol.

Human amniotic membrane was obtained from the MGH delivery room from elective Caesarian delivery patients and prepared as per protocol and frozen until required for animal surgery.

Task D - Crosslinking of HAM with EDC/NHS to make xHAM.

The processed HAM was thawed immediately before use and incubated for 1 hour in a solution of 4mM:1mM EDC:NHS to generate the durable crosslinked nerve wrap that was used for all PTB repairs, where the wrap was stained with 0.1% rose Bengal solution and photosealed over the proximal and distal neurography sites by application of 60J/cm² of green light (532 nm, 0.5W/cm²) from a CW KTP laser.

Task E - First meeting of MGH team with surgical advisors at MGH to discuss progress and suggest improvements to the research approach.

Drs. Redmond and Randolph met with Dr. Valerio at the Military Health System Research Symposium (MHSRS) conference in Kissimmee, Florida in August 2017 to discuss the behavioral testing and electrophysiological approaches used in this study and the need for immunosuppression to prevent rejection of the human-derived Avance acellular nerve allograft (ANA) in the monkeys (ultimately a xenograft material in the monkey). As a result of these discussions, the final approach was formulated.

A rigorous geometric approach to the measurement of wrist extension angle was applied and the monkeys trained as a result to reach for food treats in a specific and singular manner that prevented errors due to alternative arm movements in the animals.

Task F - Purchase and acclimatization of twenty Macaque Monkeys.

To date, all 20 male Rhesus macaque monkeys have been purchased in 3 different cohorts. The purchase and receipt schedule was determined by availability of the animals for purchase and the availability of housing space at the MGH animal facility in Boston. The schedule for receipt is shown in Table 1. All animals have now been acclimatized.

TABLE 1: Monkey schedules for the study.

NHP #	Date of Arrival	Completed Quarantine	Completed Pre-Op Training	Date of Surgery	Type of Repair	4 month Post-Op EMG
8117	09/14/18	10/23/18	01/01/18	01/08/18	autograft/suture	05/14/18
8017	09/14/18	10/23/18	01/01/18	01/19/18	autograft/suture	05/18/18
7817	09/14/18	10/23/18	01/01/18	02/05/18	Avance/PTB	06/05/18
7917	09/14/18	10/23/18	01/01/18	02/13/18	Avance/PTB	06/13/18
8217	09/14/18	10/23/18	01/01/18	03/14/18	autograft/suture	07/13/18
8317	09/14/18	10/23/18	01/01/18	03/21/18	autograft/suture	07/20/18
1118	03/26/18	05/22/18	06/09/18	06/11/18	Avance/PTB	10/11/18
1218	03/26/18	05/22/18	06/11/18	06/12/18	Avance/suture	10/12/18
818	03/26/18	05/22/18	06/17/18	06/18/18	Avance/suture	10/18/18
1018	03/26/18	05/22/18	06/17/18	06/19/18	Avance/suture	10/19/18
918	03/26/18	05/22/18	06/23/18	06/25/18	Avance/PTB	10/25/18
1018	03/26/18	05/22/18	06/23/18	06/26/18	Avance/PTB	10/26/18
X13	05/14/18	06/25/18	began 06/25/2018	07/23/18	TBD	TBD
X14	05/14/18	06/25/18	began 06/25/2018	07/24/18	TBD	TBD
X15	05/14/18	06/25/18	began 06/25/2018	08/06/18	TBD	TBD
X16	05/14/18	06/25/18	began 06/25/2018	08/07/18	TBD	TBD
X17	05/14/18	06/25/18	began 06/25/2018	08/13/18	TBD	TBD
X18	05/14/18	06/25/18	began 06/25/2018	08/14/18	TBD	TBD
X19	05/14/18	06/25/18	began 06/25/2018	08/20/18	TBD	TBD
X20	05/14/18	06/25/18	began 06/25/2018	08/21/18	TBD	TBD

Task G - Training of monkeys in behavioral task used to evaluate for functional recovery after repair.

The schedule for pre-op training of the monkeys in the task required to determine recovery of wrist extension following nerve gap injury and repair is also shown in Table 1. Acclimation of animals to research staff and facility was commenced, animals were routinely evaluated by research staff who began training animals to reach out of cage through a tube and grasp treats (mimicking the behavioral

device which was later constructed) Animals were sedated, weighed, and measurements of upper extremities obtained for construction of wrist extension device (see below).

Study staff met with MGH veterinary staff and the Environmental Enrichment Program Manager at the MGH Center for Comparative Medicine (CCM) on 9/11/2017 to discuss the equipment to be designed for longitudinal behavioral testing outcome following injury and repair of the radial nerve. As this injury affects the animal's ability to extend the wrist, a prototype was conceived where the animal must reach out the cage with the right arm, bend the elbow and reach through a tube to a box to obtain a food treat, placed on a platform that can be positioned at different heights within the enclosure, thus testing the degree to which the animal can extend the wrist. The concept is shown in Figure 1.

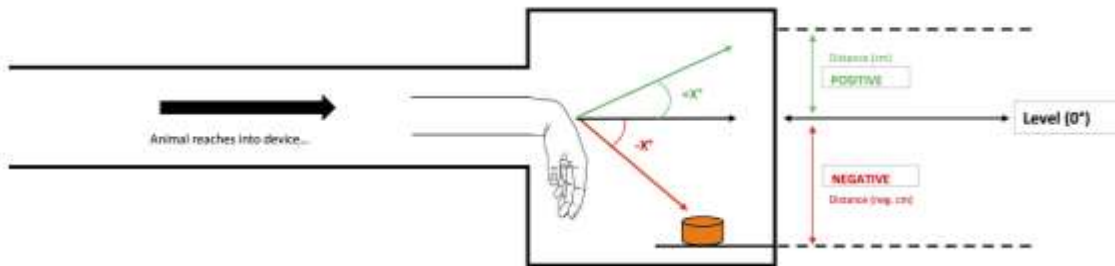


Figure 1 - schematic of behavioral testing chamber for recover of function of wrist extension

Following refinement of the design the behavioral testing device has been constructed from clear Plexiglas, allowing good visual coordination for the monkey as is shown in Figure 2. Acclimation of the animals to the final device was performed and testing of wrist extension undertaken. Pictured below is device attached to front of cage with animal reaching through tube and extending wrist in order to grasp a treat placed on shelf. Wrist angles are measured at preset platform heights as the platform can be raised until an angle is achieved where the monkey cannot extend the wrist sufficiently to grasp the food treat.



Figure 2: Actual device used for measurement of wrist extension angle.

Task H - Survival surgeries for peripheral nerve defect and repair in Rhesus monkey model.

The schedule for surgery for all 20 monkeys is shown in Table 1. The first animal was operated on 1/8/18, twelve surgeries have been performed to date and the final surgery is scheduled for 8/21/18. There has been a slight delay due to lack of availability of animals and of housing space at MGH but everything is now in place to complete the surgery schedule as planned. All surgeries have gone very well so far and the model used is excellent in terms of lack of morbidity for the animals. They retain the ability to grip and use their hands normally, with the only limitation being wrist drop in the operated arm immediately after surgery and repair of the radial nerve deficit. Aside from minor concerns on healing of the incision wound site all animals have recovered well and are well-adjusted to their environments and perform normal activities. They interact well with study staff and are more than happy to participate in the functional testing based on food treat acquisition.

Task J - Serial monthly behavioral testing for recovery of function in monkeys.

Baseline pre-op values for full range of motion, wrist extension and flexion angles were obtained through video analysis of repetitive performance of treat acquisition using a custom-designed sleeve and box apparatus with a movable platform that can be raised or lowered to specific angles to test functional performance in obtaining a food treat from the platform (Figures 1 and 2). This apparatus provides a truly objective and quantitative assessment of recovery from wrist drop following surgical repair of a 4 cm nerve deficit in the radial nerve in the right arm, affecting wrist extension. The first animals

operated are now approaching the 6 month, half-way point of the study. Table 1 also shows the repair procedures performed so far. There are 3 experimental groups. To date 5 animals have undergone repair using PTB/Avance ANA, 4 animals have received microsurgical repair with reversed autologous graft and 3 animals have received microsurgical repair with the Avance ANA. Figure 3 shows the extension angles as a function of time for the animals that have been operated so far. The first two monkeys have recently recovered a high degree of function consistent with nerve regeneration through the autologous graft in this time period.

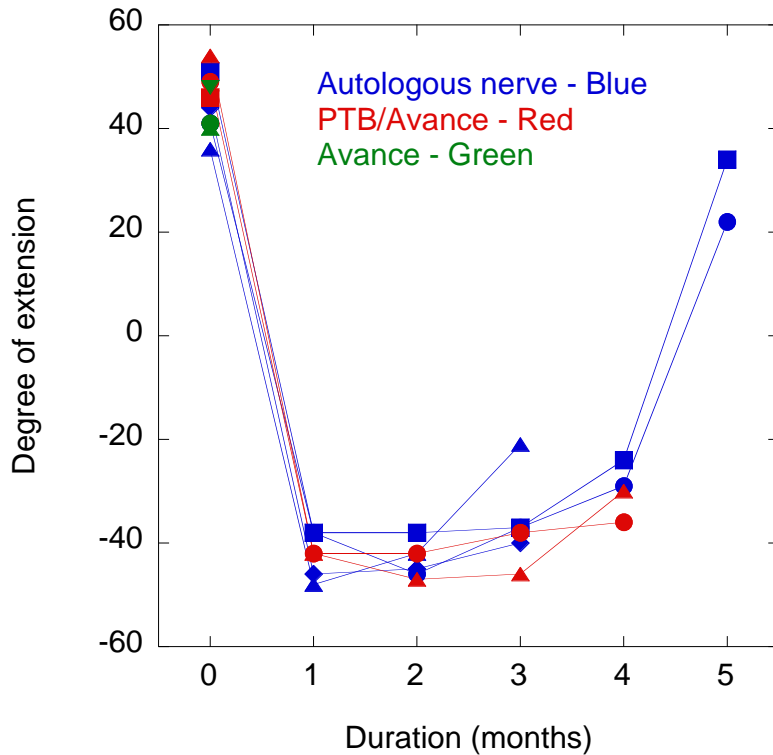


Figure 3 – Serial measurement of wrist extension angle as a function of time for each monkey.

Task K - Serial electrophysiology measurements in monkeys.

Discussions were held with Dr. William S. David MD, the Director of the EMG Laboratory and Neuromuscular Diagnostic Center at Massachusetts General Hospital, on the EMG testing, which is a component of the outcome measures for nerve regeneration in this study. Dr. David has provided valuable advice on the EMG testing and how it should be performed in this NHP model of radial nerve injury and repair. Measurements were performed under anesthesia immediately prior to surgery for each animal to determine baseline electrophysiology. CMAP values were determined for each and direct nerve recording (DNR) also performed on dissection of the radial nerve and before creation of nerve deficit. Table 1 shows the 4-month testing schedule for all animals. The first four animals in the study have also undergone the 4-month testing of CMAP, with lower values obtained as expected following injury.

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

Dr. Hansdorfer has received advanced microsurgery training from Dr. Winograd and Dr. Randolph including operating under magnification. He has also received advanced training in electrophysiology techniques from Dr. William David, Director of the EMG Laboratory and Neuromuscular Diagnostic Center at Massachusetts General Hospital.

How were the results disseminated to communities of interest?

See conference proceedings below.

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

In the next annual period we will complete all animal surgeries and continue to evaluate nerve regeneration as a function of repair method in this monkey model of nerve deficit injury. We also intend to publish the model itself for the scientific and medical research community as we feel we have devised a methodology that would be of general use in the study of surgical techniques and pharmaceutical interventions to address improvement in nerve regeneration and functional recovery in human subjects, particularly wounded warfighters and severe trauma casualties. In twelve months time we will have 60% of the animals to end point and be amassing and evaluating all the functional recovery, electrophysiology and nerve histomorphometry data to determine how advantageous the photosealing/nerve wrap approach can be towards improved outcomes for these patients.

4. Impact:

As we are midway through the study it is too early to yet say what the impact will be. However, we expect that positive results in a non-human primate model will be the final step towards demonstrating clinical efficacy and translating the technology to commercial development and clinical implementation to change the standard of care for repair of large nerve deficit injuries with optimized nerve regeneration. In addition we see this study promoting the application of photosealing for applications in orthopedics, vascular surgery, plastic surgery and GI surgery. We are in discussions with a variety of groups at present over licensing the intellectual property portfolio that surrounds our photocrosslinking technology of which nerve repair is a single but valuable component.

5. Changes/Problems:

We have had an issue in the number of NHPs that can be housed simultaneously at the MGH main campus where all surgeries will be performed. As a result of limited on-site housing the NHPs arrived and were quarantined and acclimatized at MGH Charlestown, a few miles away from the main campus. On the advice of veterinary and environmental enrichment specialists at MGH the animals are transported to the main campus two weeks in advance of surgery for acclimation and remain for two weeks following surgery before transport back to the MGH Charlestown facility. This minimizes the strain on the limited NHP housing berths at the main campus.

In addition, the animal protocol was amended for laterality of surgery due to the design of the cages used to house the animals. The opening used by the animals to obtain food is on the front right of the cage for food and necessitated a change in laterality for surgery from the left to the right arm in order to perform behavioral testing. This amendment was approved by both MGH IACUC and ACURO.

6. Products:

Invited Talk

Potential Clinical Applications of Protein Photocrosslinking. Redmond RW. Annual Meeting of the American Society for Photobiology, April 2018, Tampa, Florida.

Conference Proceedings

Light-Activated Photosealing to Optimize Outcomes of Large Gap Peripheral Nerve Defects Repaired with Acellular Nerve Grafts. Goldstein RL, Hansdorfer M, Tsui J, Visaggio M, Runyan G, Randolph MA, Meppelink AM, David WS, Winograd JM, Redmond RW. Accepted as poster presentation at the Military Health System Research Symposium 2018, Kissimee, Florida.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS:

What individuals have worked on the project?

Name: Robert W. Redmond PhD
Project Role: PI
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: 2
Contribution to Project: Dr. Redmond is responsible for overall coordination of the project

Name: Mark A. Randolph MAS
Project Role: Investigator
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: 1
Contribution to Project: Mr. Randolph has been instrumental in designing animal protocols and in the behavioral testing design.

Name: Marek Hansdorfer MD
Project Role: Research Fellow
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: 6
Contribution to Project: Dr. Hansdorfer has been the lead Fellow on this project and has been involved in all day-to day aspects of regulatory approvals, experimental planning, surgical training and behavioral testing design.

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?

Nothing to report